PLANT FRUIT WITH ELEVATED POTASSIUM LEVELS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/395,637, filed July 12, 2002, which is incorporated by reference herein in its entirety.

FIELD OF INVENTION

[0002] This invention is in the field of agricultural biotechnology. In particular, this invention relates to non-naturally occurring plants that produce fruit with elevated potassium levels when grown under conditions of elevated salt.

BACKGROUND OF THE INVENTION

[0003] Potassium is an important part of the human diet. Potassium in the diet has been shown to be beneficial to human health in a number of areas. In a recent study, people with the lowest levels of potassium in their diets were found to be 1.5 times more likely to suffer from strokes than people with the highest levels (April 13, 1998, Journal of Neurology). Increases in potassium levels in people with low potassium diets were correlated with lowered blood pressure (July 2001, Journal of Hypertension). Furthermore, diuretics may cause a person to lose potassium thus heightening the need for additional potassium in the diet. Too little potassium can negatively impact muscle tissue, especially the heart. Thus, there is a need to produce foods that have increased potassium levels.

[0004] Salt sensitive plants when grown under elevated salt conditions produce fruit with elevated levels of potassium. However, the potassium level falls off near the time of harvest and growing salt sensitive plants under elevated salt conditions involves some difficulty because the plant will die if the salt levels are too high. By contrast, naturally salt tolerant plants grown under elevated salt conditions produce fruit with levels of potassium similar to the levels produced in fruit grown under low salt. (Maria C. Bolin, et al. Plant Science 160 (2001) 1153) Thus there is a need for plants that can be grown under high salt conditions and yet still produce fruit with elevated levels of potassium.

[0005] In addition, agricultural productivity is severely affected by soil salinity, and the damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. Much research is aimed toward the breeding of crop cultivars with improved salt tolerance. One school of thought has concluded that salt tolerance will be achieved only after pyramiding several characteristics in a single genotype, where each one alone could not confer a significant increase in salt tolerance. (Yeo, et al. (1988) and Cuartero, et al. (1999)) (Full citations for the references cited herein are found after the Examples.) Arguably, salt tolerance is a complex trait, and the long list of salt stress-responsive genes seems to support this. (Zhu (2000)) The detrimental effects of salt on plants are a consequence of both a water deficit resulting in osmotic stress and the effects of excess sodium ions on key biochemical processes. In order to tolerate high levels of salts, plants should be able to utilize ions for osmotic adjustment and to internally distribute these ions to keep sodium away from the cytosol. There is thus a further need to produce salt tolerant plants. It would be particularly advantageous if the salt tolerant plants could produce fruit with elevated potassium levels since potassium is a key nutritional element as discussed above

SUMMARY OF THE INVENTION

[0006] In order to meet these needs, the present invention is directed to transgenic fruit trees, berry plants, vines and vegetables that are able to grow and produce fruit with elevated potassium levels in the presence of elevated salt concentrations. In particular, the present invention is directed to salt tolerant tomato plants that produce tomatoes with elevated potassium levels.

[0007] In one aspect, the invention is directed to a non-naturally occurring plant or plant part from said plant comprising a fruit having increased potassium levels when said plant is cultivated under elevated salt conditions. In one variations, the increased potassium levels may be at least 10% higher, at least 15% higher, at least 20% higher, at least 25% higher, at least 30% higher, at least 35% higher, at least 40% higher, at least 45% higher, or at least 50% higher. In another variation, the cultivation under elevated salt conditions may be cultivation where the elevated salt conditions persist through the

entire life cycle of the plant, the germination stage, the vegetative growth stage, the flowering stage, the seed embryogenesis stage, the stage of seed ripening, and any combination of the foregoing stages. In yet another variation, the fruit may be a flower developed fruit, an ovary developed fruit, a tomato, a grape, a strawberry, a peach, or an apple.

[0008] In another aspect, the non-naturally occurring salt tolerant plant comprises a transgene. In one variation, the transgene comprises a first nucleic acid sequence encoding a Na+/H+ transporter or a plant derived Na+/H+ transporter. In another variation, the transgene comprises a first nucleic acid selected from the following group: a nucleic acid molecule of the coding strand shown in SEQ ID NO:1, or a complement thereof; a nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO:2; a nucleic acid molecule that hybridizes to the sequence set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under highly stringent conditions that include at least one wash in 0.1xSSC, 0.1% SDS, at 65° C for thirty minutes; and a nucleic acid molecule encoding a plant NHX transporter polypeptide that hybridizes to the sequence set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under moderately stringent conditions that includes at least one wash in 0.1xSSC, 0.1% SDS, at 50° C for thirty minutes. In still another variation, the transgene further comprises a promoter sequence operably linked to the first nucleic acid sequence. In yet another variation, the promoter is a constitutive promoter or an inducible promoter. In certain variations, the promoter may be selected from the group consisting of the 35 S promoter and the CaMV promoter.

[0009] Another aspect of the present invention is a transgenic tomato comprising a first nucleic acid sequence selected from the group consisting of a nucleic acid molecule of the coding strand shown in SEQ ID NO:1, or a complement thereof; a nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO:2; a nucleic acid molecule that hybridizes to the sequence set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under highly stringent conditions that include at least one wash in 0.1xSSC, 0.1% SDS, at 65° C for thirty minutes; and a nucleic acid molecule encoding a plant NHX transporter polypeptide that hybridizes to the sequence

set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under moderately stringent conditions that includes at least one wash in 0.1xSSC, 0.1% SDS, at 50° C for thirty minutes.

[0010] An additional aspect of the present invention is a seed produced from any of the foregoing plants and variations thereof.

[0011] The present invention also includes methods of generating the foregoing. One variation includes transfecting a plant with a transcriptional regulatory element and identifying salt tolerant plants comprising a fruit having increased potassium levels when said plant is cultivated under elevated salt conditions. In another variation, plants are transfected with a transcriptional regulatory element and identifying a plant wherein said transcriptional regulatory element has integrated operably linked to a Na+/H+ transporter. In yet another variation, the transcriptional regulatory element is a promoter, an enhancer element, a repressor element or a boundary element. In one variation, plants are transfected with a transgene comprising a Na+/H+ transporter and a salt tolerant plant comprising a fruit having increased potassium levels when said plant is cultivated under elevated salt conditions is identified. In one variation, the Na+/H+ transporter gene is selected from the group consisting of a nucleic acid molecule of the coding strand shown in SEQ ID NO:1, or a complement thereof; a nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO:2; a nucleic acid molecule that hybridizes to the sequence set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under highly stringent conditions that include at least one wash in 0.1xSSC, 0.1% SDS, at 65° C for thirty minutes; and a nucleic acid molecule encoding a plant NHX transporter polypeptide that hybridizes to the sequence set forth in SEQ ID NO:1 or the complement of the sequence set forth in SEQ ID NO:1 under moderately stringent conditions that includes at least one wash in 0.1xSSC, 0.1% SDS, at 50° C for thirty minutes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **Figure 1**. Salt tolerance of wild-type tomato plants and transgenic plants overexpressing AtNHX1grown in the presence of 200 mM NaCl. (A) wild-type plants

grown in the presence of 5 mM NaCl. (B) transgenic plants overexpressing AtNHX1, grown in the presence of 5 mM NaCl. (C) Western blots from leaf membrane proteins (5 μg) tested with antibodies raised against AtNHX1. Upper panel: Lanes 1 and 4, tonoplast-enriched fraction; lanes 2 and 5, Golgi/ER-enriched fractions; 3 and 6, plasma membrane fraction. Lanes 1,2,3 correspond to membranes from wild-type plants while lanes 4,5,6 correspond to membranes from transgenic plants. Relative molecular masses are indicated on the left; lower panel: Enrichment of the fractions with tonoplast membranes was assessed with antibodies raised against the vacuolar H^+-PP_iase. (D) wild-type plants grown in the presence of 200 mM NaCl. (E)) transgenic plants overexpressing AtNHX1, grown in the presence of 200 mM NaCl. Plants shown after 11 weeks of growth.

[0013] Bar = 25 cm.

[0014] **Figure 2**. Na^+/H^+ exchange activity in leaf tonoplast vesicles Membrane fractions were purified from leaves using the method described with the modifications described. (Blumwald, et al. (1985) and Apse, et al. (1999)) At the indicated times, the vacuolar H^+-PP_iase was activated by the addition of Mg^{2+}. When a steady-state pH gradient (acidic inside) was formed, the PP_i-dependent H^+ transport activity was stopped by the addition of AMDP and the rates of cation/H^+ exchange were determined in vesicles isolated from wild-type plants (WT) and transgenic plants overexpressing AtNHX1 (X1OE). (A) Na^+-dependent H^+ exchange, (B) K^+-dependent H^+ exchange. The addition of monensin (mon), an artificial Na^+/H^+ antiport, or nigericin (nig), an artificial K^+/H^+ antiport, abolished the pH gradient and the fluorescence was fully recovered. The figure shows a typical recording.

[0015] **Figure 3**. Ion, sugar, and proline contents of wild-type and transgenic plants grown at different salt concentrations. Wild-type (hatched line bars) and transgenic plants (cross-hatched line bars) grown in the presence of 5 mM NaCl. Two independent transgenic lines (black and white bars) grown in the presence of 200 mM NaCl. (A) Na^+ contents; (B) K^+ contents; (C) Cl^- contents; (D) soluble sugar contents; (E) proline contents. For each determination, leaves, roots and fruits from ten plants were collected

from each hydroponic tank and pooled. Values are the Mean ± S.D. from material collected from three hydroponic tanks (n = 3).

[0016] **Figure 4**. Fruits from wild-type and transgenic plants. (A) tomato fruits from wild-type plants; (B) tomato fruits from transgenic plants. (C) Western blots from fruit tonoplast proteins (5 μg) tested with antibodies raised against AtNHX1. Wild-type plants grown in the presence of 5 mM NaCl (lane 1). Two independent transgenic lines grown in the presence of 200 mM NaCl (lanes 2 and 3).

DETAILED DESCRIPTION OF THE INVENTION

[0017] The present invention provides a non-naturally occurring fruit or vegetable producing plant that is characterized by producing fruit of increased potassium content. A preferred method of making such fruit or vegetable producing plant is to ectopically express a nucleic acid molecule encoding an NHX related gene product and cultivate the plant under elevated salt conditions. The NHX related gene product can have, for example, substantially the amino acid sequence of an NHX ortholog such as those described in Table II.

[0018] In one embodiment, the invention provides a transgenic fruit or vegetable producing plant characterized by producing fruit of elevated potassium content. A preferred method of producing such plant is by ectopic expression of an exogenous nucleic acid molecule encoding an NHX-related gene product. The nucleic acid molecule encoding the NHX-related gene product can be operatively linked to an exogenous regulatory element such as a constitutive regulatory element or a root, leaf or fruit-selective regulatory element.

[0019] The present invention is directed to the surprising discovery that NHX-1 regulates potassium levels in plant fruit. As disclosed herein, transgenic tomato plants over expressing an AtNHX1 were able to grow, flower and produce fruit with elevated potassium levels in the presence of 200 mM NaCl.

[0020] As further disclosed herein, overexpression of AtNHX1 in tomato results in the production of fruit having elevated potassium levels as compared to the fruit

produced by wild type tomato. As set forth in the Example constitutive expression of NHX1 under control of a 35S promoter resulted in fruit having potassium levels about 120% the amount of potassium produced in fruit of wild type plants. In view of the isolation of NHX orthologs, as detailed in Table 2, the skilled artisan will recognize that an NHX related gene product, such as an ortholog of NHX, can also be used in the methods of the present invention, for example, to produce transgenic plants having the characteristics disclosed herein. Thus, the invention provides a non-naturally occurring fruit or vegetable and plants capable of producing the same such as a transgenic tomato plant, characterized by producing fruit with elevated potassium levels due to ectopic expression of a nucleic acid molecule encoding an NHX related gene product.

[0021] The term "plant fruit," when used herein, refers to both the ovary developed fruit and the flower developed fruit. An "ovary developed fruit" is the developed ovary of a seed plant with its contents and accessory parts, as the pea pod, nut, tomato, pineapple, etc. A "flower developed fruit" is the edible part of a plant developed from a flower with any accessory tissues, as the peach, mulberry, banana, etc.

[0022] The term "elevated salt conditions," when used herein, refers to a salinity level above the highest level at which a naturally occurring plant variety can thrive and produce fruit. It is recognized that the salt tolerance of plants varies between varieties. As used herein, the naturally occurring plant variety is understood to be the same plant variety as the non-naturally occurring plant variety but for the human introduced change. One of skill in the art understands that there can be natural variation in the salt tolerance of fruit producing plants even within a variety. Thus, elevated salt conditions are those conditions above which none of a particular variety can thrive and produce fruit. Determination of elevated salt conditions is routine and in many cases for commercially relevant crop already known.

[0023] As used herein, the term "non-naturally occurring," when used in reference to a fruit or vegetable producing plant, means a seed plant that has been genetically modified by human intervention. A transgenic fruit or vegetable producing plant of the invention, for example, is a non-naturally occurring plant that contains an exogenous

nucleic acid molecule, such as a nucleic acid molecule encoding an NHX related gene product and, therefore, has been genetically modified by human intervention. In addition, a seed plant that contains, for example, a mutation in an endogenous NHX related gene product regulatory element or coding sequence as a result of calculated exposure to a mutagenic agent, such as a chemical mutagen, or an "insertional mutagen," such as a transposon, also is considered a non-naturally occurring seed plant, since it has been genetically modified by human intervention. Furthermore, a plant generated by cross breeding different strains and varieties are also considered a "non-naturally occurring plant," because the selection and breeding is performed by human intervention. In contrast, a plant containing only spontaneous or naturally occurring mutations is not a "non-naturally occurring fruit or vegetable producing plant" as defined herein and, therefore, is not encompassed within the invention. One skilled in the art understands that, while a non-naturally occurring plant typically has a nucleotide sequence that is altered as compared to a similar naturally occurring seed plant, a non-naturally occurring plant also can be genetically modified by human intervention without altering its nucleotide sequence, for example, by modifying its methylation pattern.

[0024] Based upon the above definitions, it will be clear that a "non-naturally occurring salt tolerant plant" is a plant variety that has been genetically modified by human intervention and is capable of thriving and producing fruit at elevated salt conditions, i.e., at a salinity level above which a naturally occurring plant of the same variety cannot thrive and produce fruit.

[0025] The term "ectopically," as used herein in reference to expression of a nucleic acid molecule, refers to an expression pattern in a non-naturally occurring plant that is distinct from the expression pattern in a comparable naturally occurring plant. Thus, one skilled in the art understands that ectopic expression of a nucleic acid molecule encoding an NHX-related gene product can refer to expression in a cell type other than a cell type in which the nucleic acid molecule normally is expressed, or at a time other than a time at which the nucleic acid molecule normally is expressed, or at a level other than the level at which the nucleic acid molecule normally is expressed. For example, under control of a

constitutive promoter such as the cauliflower mosaic virus 35S promoter, NHX-1 is expressed in the leaves, thus, is ectopically expressed.

[0026] The term "non-halophyte," as used herein means a plant that is not naturally morphologically and/or physiologically adapted to grow in salt rich soils or salt laden air. A non-halophyte is a plant variety that has a relative yield decrease of 50 % or more at 200 mM NaCl (the equivalent of about 20 dS/m) when compared to the plant variety grown at optimal salinity levels which are below 200 mM NaCl. The invention is suitable for even more salt sensitive plant varieties which have a relative yield decrease of 50% or more at 180 mM NaCl, 160 mM NaCl, 140 mM NaCl, 120 mM NaCl, 100 mM NaCl or 80 mM NaCl. Table IV lists the relative yield decrease for various non-halophyte crop plants.

[0027] The term "saline-intolerant plants" as used herein means a plant variety that cannot complete its life cycle in growth media containing a salinity level above 200 mM NaCl. The invention is suitable for even more highly saline-intolerant plant varieties that cannot complete their life cycle in growth media containing a salinity level above 180 mM NaCl, 160 mM NaCl, 140 mM NaCl, 120 mM NaCl, 100 mM NaCl and even 7 mM NaCl.

Increased Potassium Levels

[0028] The term "increased potassium levels," as used herein in reference to a fruit or vegetable produced by a non-naturally occurring berry plant or bush, fruit or vegetable producing plant varieties of the invention, means higher potassium levels when grown at elevated salt conditions as compared to the potassium levels of fruit or vegetables produced by a corresponding plant variety lacking a genetic modification introduced by human intervention such as an ectopically expressed nucleic acid molecule encoding an NHX related gene product such as a wild type plant. As disclosed herein in the Example, the seeds from a transgenic tomato plant ectopically expressing NHX-1 produce fruit that have potassium levels exhibiting almost 120% of the potassium levels of fruit produced from wild type tomato plants when grown under 200 mM NaCl.

[0029] It is recognized that there can be natural variation in the potassium levels of fruit or vegetables produced by a particular plant species or variety. However, fruit of increased potassium levels produced by a plant using a method of the invention readily can be identified by sampling a population of the produced fruit or vegetables and determining that the normal potassium distribution of fruit or vegetable is greater, on average, than the normal distribution of fruit or vegetables produced by the corresponding plant variety or species lacking a genetic modification introduced by human intervention such as an ectopically expressed nucleic acid molecule encoding an NHX related gene product. Thus, production of non-naturally occurring plants of the invention provides a means to skew the normal distribution of fruit or vegetable potassium levels produced by a plant, such that the fruit or vegetable potassium levels are, on average, at least about 5% greater, 10% greater, 15% greater, 20% greater, 25% greater, 30% greater, 50% greater, 75% greater, 100% greater, 200% greater, 300% greater, 400% greater or 500% greater than in the corresponding plant species that does not contain a genetic modification introduced by human intervention such as an ectopically expressed nucleic acid molecule encoding an NHX related gene product.

[0030] As used herein, the term "NHX-related gene product" means a gene product that has the same or similar function as At NHX-1 such that, when ectopically expressed in a plant, normal development is altered such that fruit or vegetables of increased potassium levels are produced. Arabidopsis NHX-1 is an example of an NHX related gene product as defined herein.

[0031] An NHX related gene product generally is characterized, in part, as containing a putative cation binding domain and an amiloride binding domain. An NHX-1 related gene product also generally is characterized by having an amino acid sequence that has at least about 40% amino acid identity with the amino acid sequence of Arabidopsis NHX-1. An NHX related gene product can have, for example, an amino acid sequence with greater than about 45% amino acid sequence identity with Arabidopsis NHX-1, preferably greater than about 50% amino acid identity with Arabidopsis NHX-1, preferably greater than about 55% amino acid sequence identity with Arabidopsis NHX-1, preferably greater than about 60% amino acid identity with Arabidopsis NHX-1,

preferably greater than about 65% amino acid sequence identity with Arabidopsis NHX-1, preferably greater than about 75% amino acid identity with Arabidopsis NHX-1, more preferably greater than about 85% amino acid identity with Arabidopsis NHX-1, and can be a sequence having greater than about 90%, 95% or 97% amino acid identity with Arabidopsis NHX-1.

[0032] Preferably, an NHX-related gene product is orthologous to the plant species in which it is ectopically expressed. A nucleic acid molecule encoding tomato NHX, for example, can be ectopically expressed in a tomato plant to produce a non-naturally occurring tomato variety characterized by producing tomatoes with increased potassium levels. Similarly, a nucleic acid molecule encoding fruit tree NHX, for example, can be ectopically expressed in a fruit tree to produce a non-naturally occurring fruit tree characterized by producing fruit with increased potassium levels.

[0033] A nucleic acid molecule encoding an NHX-related gene product also can be ectopically expressed in a heterologous plant to produce a non-naturally occurring plant characterized by producing fruit with elevated potassium levels. NHX proteins have been cloned from a number of plant species (including Arabidopsis, tomato, sugar beets, petunia, rice, etc). indicating that they are widely conserved throughout the plant species. NHX-related gene products such as NHX orthologs also can be conserved and can function across species boundaries to produce fruit with increased potassium levels. Thus, ectopic expression of a nucleic acid molecule encoding an NHX-related gene product in a heterologous plant can alter fruit potassium levels. Furthermore, a nucleic acid molecule encoding an NHX-related gene product, for example, can be ectopically expressed in more distantly related heterologous plants, including dicotyledonous and monocotyledonous angiosperms and gymnosperms, fruit trees, berry plants and vines and, upon ectopic expression, can alter fruit potassium levels.

[0034] As used herein, the term "NHX-related gene product" encompasses an active segment of an NHX-related gene product, which is a polypeptide portion of an NHX-related gene product that, when ectopically expressed, increases fruit potassium levels. An active segment can be, for example, an amino terminal, internal or carboxy terminal

fragment of Arabidopsis NHX-1 that, when ectopically expressed in a plant, produces fruit with elevated potassium levels. The skilled artisan will recognize that a nucleic acid molecule encoding an active segment of an NHX-related gene product can be used to generate a plant of the invention characterized by producing fruit with elevated potassium levels and in the related methods and kits of the invention described further below.

[0035] An active segment of an NHX-related gene product can be identified using the methods described in the Example or using other routine methodology. Briefly, a seed plant such as tomato can be transformed with a nucleic acid molecule under control of a constitutive regulatory element such as a tandem CaMV 35S promoter. Biochemical analysis of the plant reveals whether a seed plant ectopically expressing a particular polypeptide portion produces fruit with elevated potassium levels. For analysis of a large number of polypeptide portions of an NHX-related gene product, nucleic acid molecules encoding the polypeptide portions can be assayed in pools, and active pools subsequently subdivided to identify the active nucleic acid molecule.

[0036] In one embodiment, the invention provides a non-naturally occurring seed plant that is characterized by producing fruit with elevated potassium levels due to ectopic expression of a nucleic acid molecule encoding an NHX-related gene product having substantially the amino acid sequence of an NHX ortholog. As used herein, the term "NHX ortholog" means an ortholog of Arabidopsis NHX-1 and refers to an NHX-related gene product that, in a particular plant variety, has the highest percentage homology at the amino acid level to Arabidopsis NHX-1. An NHX-1 ortholog can be, for example the NHX-1 orthologs described in Table 2. Novel NHX ortholog cDNAs can be isolated from additional plant species using a nucleotide sequence as a probe and methods well known in the art of molecular biology (Glick and Thompson (eds.), Methods in Plant Molecular Biology and Biotechnology, Boca Raton, Fla.: CRC Press (1993); Sambrook et al. (eds.), Molecular Cloning: A Laboratory Manual (Second Edition), Plainview, N.Y.: Cold Spring Harbor Laboratory Press (1989), each of which is incorporated herein by reference).

[0037] As used herein, the term "substantially the amino acid sequence," when used in reference to an NHX ortholog, is intended to mean a polypeptide or polypeptide segment having an identical amino acid sequence, or a polypeptide or polypeptide segment having a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an NHX-related gene product having substantially the amino acid sequence of Arabidopsis NHX-1 can have an amino acid sequence identical to the sequence of Arabidopsis NHX-1, or a similar, non-identical sequence that is functionally equivalent. In particular, a gene product that has "substantially the amino acid sequence" of an NHX ortholog can have one or more modifications such as amino acid additions, deletions or substitutions, including conservative or non-conservation substitutions, relative to the NHX-1 amino acid sequence, for example, provided that the modified polypeptide retains substantially the ability to increase fruit potassium levels when the nucleic acid molecule is ectopically expressed in the plant. Comparison of sequences for substantial similarity can be performed between two sequences of any length and usually is performed with sequences between about 6 and 1200 residues, preferably between about 10 and 100 residues and more preferably between about 25 and 35 residues. Such comparisons for substantial similarity are performed using methodology routine in the art.

[0038] The preferred percentage of sequence similarity for sequences of NHX orthologs includes nucleotide sequences having at least about: 48% similarity to SEQ ID NO:1. The similarity may also be at least about: 60% similarity, 75% similarity, 80% similarity, 90% similarity, 95% similarity, 97% similarity, 98% similarity, 99% similarity, or more preferably at least about 99.5% similarity, wherein the polypeptide has Na+/H+ transporter activity. The invention also includes salt tolerant plants made by transgenic expression of nucleic acid molecules encoding polypeptides, with the polypeptides having at least about: at least about: 48% similarity to SEQ ID NO:2. The similarity may also be at least about: 60% similarity, 75% similarity, 80% similarity, 90% similarity, 95% similarity, 97% similarity, 98% similarity, 99% similarity, or more preferably at least about 99.5% similarity, wherein the polypeptide Na+/H+ has transporter activity, to SEQ ID NO:2 (or a partial sequence thereof) considering conservative amino acid changes, wherein the polypeptide has Na+/H+ transporter

activity. Sequence similarity is preferably calculated as the number of similar amino acids in a pairwise alignment expressed as a percentage of the shorter of the two sequences in the alignment. The pairwise alignment is preferably constructed using the Clustal W program, using the following parameter settings: fixed gap penalty=10, floating gap penalty=10, protein weight matrix=BLOSUM62. Similar amino acids in a pairwise alignment are those pairs of amino acids which have positive alignment scores defined in the preferred protein weight matrix (BLOSUM62). The protein weight matrix BLOSUM62 is considered appropriate for the comparisons described here by those skilled in the art of bioinformatics. (The reference for the clustal w program (algorithm) is Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680; and the reference for BLOSUM62 scoring matrix is Henikoff, S. and Henikoff, J.G. (1993) Performance evaluation of amino acid substitution matrices. Proteins, 7:49-61.)

[0039] It is understood that minor modifications of primary amino acid sequence can result in an NHX-related gene product that has substantially equivalent or enhanced function as compared to the NHX ortholog from which it was derived. Further, various molecules can be attached to an NHX ortholog or active segment thereof, for example, other polypeptides, antigenic or other peptide tags, carbohydrates, lipids, or chemical moieties. Such modifications are included within the term NHX ortholog as defined herein.

[0040] One or more point mutations can be introduced into a nucleic acid molecule encoding an NHX ortholog to yield a modified nucleic acid molecule using, for example, site-directed mutagenesis (see Wu (Ed.), Meth. In Enzymol. Vol. 217, San Diego: Academic Press (1993); Higuchi, "Recombinant PCR" in Innis et al. (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990), each of which is incorporated herein by reference). Such mutagenesis can be used to introduce a specific, desired amino acid insertion, deletion or substitution; alternatively, a nucleic acid sequence can be synthesized having random nucleotides at one or more predetermined positions to

generate random amino acid substitutions. Scanning mutagenesis also can be useful in generating a modified nucleic acid molecule encoding substantially the amino acid sequence of an NHX ortholog.

[0041] Modified nucleic acid molecules can be routinely assayed for the ability to alter normal plant development such that fruit with elevated potassium levels are produced. For example, a nucleic acid molecule encoding substantially the amino acid sequence of an NHX ortholog can be ectopically expressed, for example, using a constitutive regulatory element such as the CaMV 35S promoter or using a tissue-specific regulatory element such as a fruit-selective regulatory element as described further below. If such ectopic expression results in a plant in which fruit or vegetables of elevated potassium levels are produced, the modified polypeptide or segment is an "NHX ortholog" as defined herein.

[0042] Other functional equivalent forms of the NHX-related gene product encoding nucleic acids can be identified using conventional DNA-DNA or DNA-RNA hybridization techniques. These nucleic acid molecules and the AtNHX sequences can be modified without significantly affecting their activity.

[0043] The plants of the present invention may therefore also be made by generating transgenic plants containing nucleic acid molecules that hybridize to one SEQ ID NO:1 or their complementary sequences, and that encode expression for peptides or polypeptides exhibiting substantially equivalent activity as that of an AtNHX polypeptide produced by SEQ ID NO:1 or their variants. Such nucleic acid molecules preferably hybridize to the sequences under low, moderate (intermediate), or high stringency conditions. (see Sambrook et al. (Most recent edition) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

[0044] As used herein, the phrase "low stringency hybridization conditions" refers the following conditions and equivalents thereto: hybridization at 5xSSC, 2% SDS, and 100 μg/ml single stranded DNA at 40° C for 8 hours, followed by at least one wash in 2xSSC, 0.2% SDS, at 40° C for thirty minutes.

[0045] As used herein, the phrase "moderate stringency hybridization conditions" refers the following conditions and equivalents thereto: hybridization at 5xSSC, 2% SDS, and 100 μg/ml single stranded DNA at 50° C for 8 hours, followed by at least one wash in 0.1xSSC, 0.1% SDS, at 50° C for thirty minutes.

[0046] As used herein, the phrase "high stringency hybridization conditions" refers the following conditions and equivalents thereto: hybridization at 5xSSC, 2% SDS, and 100 μg/ml single stranded DNA at 65° C for 8 hours, followed by at least one wash in 0.1xSSC, 0.1% SDS, at 65° C for thirty minutes.

[0047] A non-naturally occurring plant of the invention that is characterized by producing fruit with elevated potassium levels can be one of a variety of plant species, including a monocotyledonous or dicotyledonous angiosperm or a gymnosperm.

[0048] The invention also provides a transgenic plant that is characterized by producing fruit with elevated potassium levels. A preferred method of making such a transgenic plant is by ectopic expression of an exogenous nucleic acid molecule encoding an NHX-related gene product. In a transgenic plant of the invention, the ectopically expressed exogenous nucleic acid molecule encoding an NHX-related gene product can be operatively linked to an exogenous regulatory element. In one embodiment, the invention provides a transgenic plant characterized by producing fruit with elevated potassium levels having an ectopically expressed exogenous nucleic acid molecule encoding an NHX-related gene product that is operatively linked to a constitutive regulatory element. The invention provides, for example, a transgenic plant that is characterized by producing fruit with elevated potassium levels due to ectopic expression of an exogenous nucleic acid molecule encoding substantially the amino acid sequence of an NHX ortholog operatively linked to a cauliflower mosaic virus 35S promoter.

[0049] In another embodiment, an exogenous constitutive or inducible regulatory element may be introduced to the plant such that the exogenous regulatory element is operably linked to an endogenous gene and alters the expression pattern of the gene in a manner that elevates the potassium level in the fruit. One example of this would be to transfect a plant with the cauliflower mosaic virus 35S promoter such that the promoter

integrates in a way that it is operably linked to one of the plant's endogenous NHX-related genes.

[0050] In yet another embodiment, an exogenous NHX-related gene may be introduced to the plant such that the exogenous NHX-related gene is operably linked to an endogenous regulatory element which directs the expression of the gene in a manner that elevates the potassium level in the fruit.

[0051] Yet another embodiment is to transfect a plant with an NHX-related gene with out a promoter in such a way that it integrates operably linked to an endogenous promoter in the plant. One example of this would be to transfect a plant with the atNHX1 gene such that the gene integrates in a way that it is operably linked to one of the plant's endogenous strong promoters.

[0052] As used herein, the term "transgenic" refers to a seed plant that contains an exogenous nucleic acid molecule, which can be derived from the same plant species or from a heterologous plant species.

[0053] The term "exogenous," as used herein in reference to a nucleic acid molecule and a transgenic plant, means a nucleic acid molecule originating from outside the plant. An exogenous nucleic acid molecule can have a naturally occurring or non-naturally occurring nucleotide sequence. One skilled in the art understands that an exogenous nucleic acid molecule can be a heterologous nucleic acid molecule derived from a different plant species than the plant into which the nucleic acid molecule is introduced or can be a nucleic acid molecule derived from the same plant species as the seed plant into which it is introduced.

[0054] The term "operatively linked," as used in reference to a regulatory element and a nucleic acid molecule, such as a nucleic acid molecule encoding an NHX-related gene product, means that the regulatory element confers regulated expression upon the operatively linked nucleic acid molecule. Thus, the term "operatively linked," as used in reference to an exogenous regulatory element such as a constitutive regulatory element and a nucleic acid molecule encoding an NHX-related gene product, means that the

constitutive regulatory element is linked to the nucleic acid molecule encoding an NHX-related gene product such that the expression pattern of the constitutive regulatory element is conferred upon the nucleic acid molecule encoding the NHX-related gene product. It is recognized that a regulatory element and a nucleic acid molecule that are operatively linked have, at a minimum, all elements essential for transcription, including, for example, a TATA box.

Constitutive Regulatory Elements

[0055] As used herein, the term "constitutive regulatory element" means a regulatory element that confers a level of expression upon an operatively linked nucleic molecule that is relatively independent of the cell or tissue type in which the constitutive regulatory element is expressed. A constitutive regulatory element that is expressed in a plant generally is widely expressed in a large number of cell and tissue types.

[0056] A variety of constitutive regulatory elements useful for ectopic expression in a transgenic plant of the invention are well known in the art. The cauliflower mosaic virus 35S (CaMV 35S) promoter, for example, is a well-characterized constitutive regulatory element that produces a high level of expression in all plant tissues (Odell et al., Nature 313:810-812 (1985)). The CaMV 35S promoter can be particularly useful due to its activity in numerous diverse plant species (Benfey and Chua, Science 250:959-966 (1990); Futterer et al., Physiol. Plant 79:154 (1990); Odell et al., supra, 1985). A tandem 35S promoter, in which the intrinsic promoter element has been duplicated, confers higher expression levels in comparison to the unmodified 35S promoter (Kay et al., Science 236:1299 (1987)). Other constitutive regulatory elements useful for ectopically expressing a nucleic acid molecule encoding an NHX-related gene product in a transgenic seed plant of the invention include, for example, the cauliflower mosaic virus 19S promoter; the Figwort mosaic virus promoter; and the nopaline synthase (nos) gene promoter (Singer et al., Plant Mol. Biol. 14:433 (1990); An, Plant Physiol. 81:86 (1986)).

[0057] Additional constitutive regulatory elements including those for efficient ectopic expression in monocots also are known in the art, for example, the pEmu promoter and promoters based on the rice Actin-1 5' region (Last et al., Theor. Appl.

Genet. 81:581 (1991); Mcelroy et al., Mol. Gen. Genet. 231:150 (1991); Mcelroy et al., Plant Cell 2:163 (1990)). Chimeric regulatory elements, which combine elements from different genes, also can be useful for ectopically expressing a nucleic acid molecule encoding an NHX-related gene product (Comai et al., Plant Mol. Biol. 15:373 (1990)). One skilled in the art understands that a particular constitutive regulatory element is chosen based, in part, on the plant species in which a nucleic acid molecule encoding an NHX-related gene product is to be ectopically expressed and on the desired level of expression.

[0058] An exogenous regulatory element useful in a transgenic plant of the invention also can be an inducible regulatory element, which is a regulatory element that confers conditional expression upon an operatively linked nucleic acid molecule, where expression of the operatively linked nucleic acid molecule is increased in the presence of a particular inducing agent or stimulus as compared to expression of the nucleic acid molecule in the absence of the inducing agent or stimulus. Particularly useful inducible regulatory elements include copper-inducible regulatory elements (Mett et al., Proc. Natl. Acad. Sci. USA 90:4567-4571 (1993); Furst et al., Cell 55:705-717 (1988)); tetracycline and chlor-tetracycline-inducible regulatory elements (Gatz et al., Plant J. 2:397-404 (1992); Roder et al., Mol. Gen. Genet. 243:32-38 (1994); Gatz, Meth. Cell Biol. 50:411-424 (1995)); ecdysone inducible regulatory elements (Christopherson et al., Proc. Natl. Acad. Sci. USA 89:6314-6318 (1992); Kreutzweiser et al., Ecotoxicol. Environ. Safety 28:14-24 (1994)); heat shock inducible regulatory elements (Takahashi et al., Plant Physiol. 99:383-390 (1992); Yabe et al., Plant Cell Physiol. 35:1207-1219 (1994); Ueda et al., Mol. Gen. Genet. 250:533-539 (1996)); and lac operon elements, which are used in combination with a constitutively expressed lac repressor to confer, for example, IPTG-inducible expression (Wilde et al., EMBO J. 11:1251-1259 (1992)).

[0059] An inducible regulatory element useful in the transgenic seed plants of the invention also can be, for example, a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al., Plant Mol. Biol. 17:9 (1991)) or a light-inducible promoter, such as that associated with the small subunit of RuBP carboxylase or the LHCP gene families (Feinbaum et al., Mol. Gen. Genet. 226:449 (1991); Lam and Chua,

Science 248:471 (1990)). Additional inducible regulatory elements include salicylic acid inducible regulatory elements (Uknes et al., Plant Cell 5:159-169 (1993); Bi et al., Plant J. 8:235-245 (1995)); plant hormone-inducible regulatory elements (Yamaguchi-Shinozaki et al., Plant Mol. Biol. 15:905 (1990); Kares et al., Plant Mol. Biol. 15:225 (1990)); and human hormone-inducible regulatory elements such as the human glucocorticoid response element (Schena et al., Proc. Natl. Acad. Sci. USA 88:10421 (1991)).

[0060] It should be recognized that a non-naturally occurring plant of the invention, which contains an ectopically expressed nucleic acid molecule encoding an NHX-related gene product, also can contain one or more additional modifications, including naturally and non-naturally occurring mutations that can, for example, increase fruit potassium levels.

[0061] The invention further provides a method of producing a non-naturally occurring plant characterized by producing fruit with elevated potassium levels. One method is practiced by ectopically expressing a nucleic acid molecule encoding an NHX-related gene product in the plant, whereby fruit potassium levels are increased due to ectopic expression of the nucleic acid molecule. In one embodiment, the method is practiced by introducing an exogenous nucleic acid molecule encoding an NHX-related gene product into the plant.

[0062] As discussed above, the term "ectopically" refers to expression of a nucleic acid molecule encoding an NHX-related gene product in a cell type other than a cell type in which the nucleic acid molecule is normally expressed, at a time other than a time at which the nucleic acid molecule is normally expressed or at an expression level other than the level at which the nucleic acid molecule normally is expressed.

[0063] Actual ectopic expression of an NHX-related gene product is dependent on various factors. The ectopic expression can be widespread expression throughout most or all plant tissues or can be expression restricted to a small number of plant tissues, and can be achieved by a variety of routine techniques. Mutagenesis, including seed or pollen mutagenesis, can be used to generate a non-naturally occurring seed plant, in which a

nucleic acid molecule encoding an NHX-related gene product is ectopically expressed. Ethylmethane sulfonate (EMS) mutagenesis, transposon mediated mutagenesis or T-DNA mediated mutagenesis also can be useful in ectopically expressing an NHX-related gene product to produce a seed plant that produces seeds of increased size (see, generally, Glick and Thompson, supra, 1993). While not wishing to be bound by any particular mechanism, ectopic expression in a mutagenized plant can result from inactivation of one or more negative regulators of NHX, for example.

[0064] Ectopic expression of an NHX-related gene product also can be achieved by expression of a nucleic acid molecule encoding an NHX-related gene product from a heterologous regulatory element or from a modified variant of its own promoter. Heterologous regulatory elements include constitutive regulatory elements, which result in expression of the NHX-related gene product in the fruit as well as in a variety of other cell types, and seed-selective regulatory elements, which produce selective expression of an NHX-related gene product in a limited number of plant tissues, including one or more fruit tissues.

[0065] Ectopic expression of a nucleic acid molecule encoding an NHX-related gene product can be achieved using an endogenous or exogenous nucleic acid molecule encoding an NHX-related gene product. A recombinant exogenous nucleic acid molecule can contain a heterologous regulatory element that is operatively linked to a nucleic acid sequence encoding an NHX-related gene product. Methods for producing the desired recombinant nucleic acid molecule under control of a heterologous regulatory element and for producing a non-naturally occurring plant of the invention are well known in the art (see, generally, Sambrook et al., supra, 1989; Glick and Thompson, supra, 1993).

Transformation

[0066] An exogenous nucleic acid molecule can be introduced into a plant for ectopic expression using a variety of transformation methodologies including Agrobacterium-mediated transformation and direct gene transfer methods such as electroporation and microprojectile-mediated transformation (see, generally, Wang et al. (eds), Transformation of Plants and Soil Microorganisms, Cambridge, UK: University Press

(1995), which is incorporated herein by reference). Transformation methods based upon the soil bacterium Agrobacterium tumefaciens are particularly useful for introducing an exogenous nucleic acid molecule into a seed plant. The wild type form of Agrobacterium contains a Ti (tumor-inducing) plasmid that directs production of tumorigenic crown gall growth on host plants. Transfer of the tumor-inducing T-DNA region of the Ti plasmid to a plant genome requires the Ti plasmid-encoded virulence genes as well as T-DNA borders, which are a set of direct DNA repeats that delineate the region to be transferred. An Agrobacterium-based vector is a modified form of a Ti plasmid, in which the tumor inducing functions are replaced by the nucleic acid sequence of interest to be introduced into the plant host.

[0067] Agrobacterium-mediated transformation generally employs cointegrate vectors or, preferably, binary vector systems, in which the components of the Ti plasmid are divided between a helper vector, which resides permanently in the Agrobacterium host and carries the virulence genes, and a shuttle vector, which contains the gene of interest bounded by T-DNA sequences. A variety of binary vectors are well known in the art and are commercially available, for example, from Clontech (Palo Alto, Calif.). Methods of coculturing Agrobacterium with cultured plant cells or wounded tissue such as leaf tissue, root explants, hypocotyledons, stem pieces or tubers, for example, also are well known in the art (Glick and Thompson, supra, 1993). Wounded cells within the plant tissue that have been infected by Agrobacterium can develop organs de novo when cultured under the appropriate conditions; the resulting transgenic shoots eventually give rise to transgenic plants that ectopically express a nucleic acid molecule encoding an NHX-related gene product. Agrobacterium also can be used for transformation of whole seed plants as described in Bechtold et al., C.R. Acad. Sci. Paris. Life Sci. 316:1194-1199 (1993), (which is incorporated herein by reference). Agrobacterium-mediated transformation is useful for producing a variety of transgenic seed plants (Wang et al., supra, 1995) including transgenic plants of the Brassicaceae family, such as rapeseed and flax, and transgenic plants of the Fabaceae family such as soybean, pea, lentil and bean.

[0068] Microprojectile-mediated transformation also can be used to produce a transgenic seed plant that ectopically expresses an NHX-related gene product. This

method, first described by Klein et al. (Nature 327:70-73 (1987), which is incorporated herein by reference), relies on microprojectiles such as gold or tungsten that are coated with the desired nucleic acid molecule by precipitation with calcium chloride, spermidine or PEG. The microprojectile particles are accelerated at high speed into an angiosperm tissue using a device such as the BIOLISTIC PD-1000 (Biorad; Hercules Calif.).

[0069] Microprojectile-mediated delivery or "particle bombardment" is especially useful to transform seed plants that are difficult to transform or regenerate using other methods. Microprojectile-mediated transformation has been used, for example, to generate a variety of transgenic plant species, including cotton, tobacco, corn, hybrid poplar and papaya (see Glick and Thompson, supra, 1993) as well as cereal crops such as wheat, oat, barley, sorghum and rice (Duan et al., Nature Biotech. 14:494-498 (1996); Shimamoto, Curr. Opin. Biotech. 5:158-162 (1994), each of which is incorporated herein by reference). In view of the above, the skilled artisan will recognize that Agrobacterium-mediated or microprojectile-mediated transformation, as disclosed herein, or other methods known in the art can be used to produce a transgenic seed plant of the invention.

Kits

[0070] Kits for generating a transgenic plant characterized by producing fruit of elevated potassium levels are provided herein. The kits of the invention include a nucleic acid molecule encoding an NHX-related gene product and a regulatory element. In a kit of the invention, the NHX-related gene product can have, for example, substantially the amino acid sequence of an NHX ortholog. If desired, a kit for generating a transgenic plant characterized by producing fruit of elevated potassium levels can include a plant expression vector containing a nucleic acid molecule encoding an NHX-related gene product operatively linked to a seed-selective regulatory element.

[0071] Nucleic acid molecules encoding NHX-related gene products, such as those having substantially the amino acid sequence of an NHX ortholog, have been described hereinabove. A kit of the invention can contain one of a variety of nucleic acid

molecules encoding NHX-related gene products and any regulatory element, such as an element described hereinabove.

[0072] If desired, a kit of the invention also can contain a plant expression vector. As used herein, the term "plant expression vector" means a self-replicating nucleic acid molecule that provides a means to transfer an exogenous nucleic acid molecule into a seed plant host cell and to express the molecule therein. Plant expression vectors encompass vectors suitable for Agrobacterium-mediated transformation, including binary and cointegrating vectors, as well as vectors for physical transformation.

[0073] Plant expression vectors can be used for transient expression of the exogenous nucleic acid molecule, or can integrate and stably express the exogenous sequence. One skilled in the art understands that a plant expression vector can contain all the functions needed for transfer and expression of an exogenous nucleic acid molecule; alternatively, one or more functions can be supplied in trans as in a binary vector system for Agrobacterium-mediated transformation.

[0074] In addition to containing a nucleic acid molecule encoding an NHX-related gene product operatively linked to a seed-selective regulatory element, a plant expression vector of the invention can contain, if desired, additional elements. A binary vector for Agrobacterium-mediated transformation contains one or both T-DNA border repeats and can also contain, for example, one or more of the following: a broad host range replicon, an ori T for efficient transfer from E. coli to Agrobacterium, a bacterial selectable marker such as ampicillin and a polylinker containing multiple cloning sites.

[0075] A plant expression vector for physical transformation can have, if desired, a plant selectable marker and can be based on a vector such as pBR322, pUC, pGEM and M13, which are commercially available, for example, from Pharmacia (Piscataway, N.J.) or Promega (Madison, Wis.). In plant expression vectors for physical transformation of a seed plant, the T-DNA borders or the ori T region can optionally be included but provide no advantage.

[0076] The invention also provides a method of generating a non-naturally occurring plant that is characterized by producing fruit or vegetables of increased potassium levels. The method includes the step of ectopically expressing a nucleic acid molecule encoding an NHX-family gene product in the plant, whereby fruit potassium levels are increased due to ectopic expression of the nucleic acid molecule. In one embodiment, the method includes the step of introducing an exogenous nucleic acid molecule encoding an NHX-family gene product into the plant.

[0077] Examples of a non-naturally occurring seed plant of the invention characterized by producing fruit of increased potassium levels include vegetables such as tomatoes, citrus trees, such as orange trees, grapefruit trees, lemon trees and lime trees. A non-naturally occurring plant of the invention characterized by producing fruit of increased potassium level also can be a plant that bears, for example, grapes, apples, pears, peaches, plums, cherries, bananas, blackberries, blueberries, raspberries, strawberries, pineapples, dates, avocados, olives, tomatoes, cucumbers or eggplants, such fruits having an increased potassium level as compared to the fruit produced by the corresponding wild type plant.

[0078] The invention will be better understood by reference to the following non-limiting example.

EXAMPLE

Experimental Protocol

Plant Material and transgenic plants.

[0079] Lycopersicon esculentum (cv Moneymaker) seeds were germinated on Murashige and Skoog medium (MS). Cotyledon explants were excised from 7 day-old seedlings, cut in half and cultured overnight on a one day-old feeder layer consisting of 3 ml of a 7 day-old sugar beet suspension culture plated and overlaid with a sterile Whatman filter paper. The binary Ti vector pBI121 was used for transformation. The GUS gene26 of the binary vector was replaced with the AtNHX1 gene to gain the new expression construct pHZX1. pHZX1 was electroporated into Agrobacterium tumefaciens strain LBA4404. For co-cultivation, 1 ml of pHZX1 containing Agrobacterium were inoculated into 15 ml LB medium containing 50 mg/l kanamycin, 50 mg/l rifampicin and 200 μM acetone-syringone. After two days of co-cultivation with Agrobacterium, the explants were transferred to selective regeneration medium 27. Regenerated shoots were transferred to fresh medium bi-weekly. When the green shoots were 1-2 cm tall, they were separated from the calli and transferred onto rooting medium containing modified MS salts27. About 98% shoots can form roots in two weeks. Rooted shoots were transplanted to soil and plants regenerated. T1 seeds were grown on plates containing MS medium and 100 mg/l kanamycin and homozygous seeds selected.

[0080] For salt tolerance experiments, wild type and two independent lines (T2) of transgenic plants were grown hydroponically. Seeds were germinated in agar plates containing MS medium under continuous light at 25 °C. Two weeks after germination, sixty of each wild-type and transgenic seedlings were transferred to six hydroponic tanks, containing 20 seedlings each tank, and grown in the greenhouse. Day temperature was maintained at 26 ± 2 °C and night temperature was 22 ± 2 °C. Relative humidity was maintained at 50 ±10%. Plants were grown under a 14 h/10 h light/dark photoperiod. Supplemental lighting consisted of eight high-pressure sodium lamps, and resulted in a total (sunlight and supplemental light) of approximately 1,250 μmol/m2s. The nutrient solution was obtained by mixing 1.2 g per liter of stock fertilizer (tomato fertilizer, Plant-

Prod, Brampton, Ontario) and 1g per liter of $CaNO_3$. The final nutrient solution contained (in mg/l) 200 N, 54 P, 256 K, 147 Ca, 42 Mg, micronutrients and was supplemented with 5 mM or 200 mM NaCl. The nutrient solution was replaced every 6 days and the roots were kept under constant aeration.

Membrane isolation and Western blots.

[0081] Membrane fractions were isolated from shoots of 4-week-old plants or tomato fruits from mature plants as described 5. Western blots of the different membrane fractions were performed as described4.

Transport assays.

[0082] The cation/H^+ exchange activity was measured by following the pH dependent fluorescence quenching of acridine orange5. An acidic-inside pH gradient across the tonoplast vesicles was obtained by activation of the vacuolar H^+-PPiase. Twenty μg of tonoplast vesicles were added to 0.8 ml buffer containing 0.25 M Mannitol, 5 mM Tris/MES (pH 8.0), 2 mM dithiotreitol, 25 mM KCl, 0.8 mM Tris-PPi and 5 μM acridine orange. Proton translocation was initiated by the addition of 1 mM Mg^{2+} and the change in fluorescence was monitored as described5. When a steady-state pH gradient (acidic inside) was formed, PPi-dependent H^+-transport activity was stopped by the addition of AMDP and the changes in rate of fluorescence recovery were determined in the presence and absence of 50 mM NaCl.

Leaf and fruit chemical analysis.

[0083] Chemical analysis from 3-month old plants was performed. Fully-expanded mature leaves from the six most lower basal nodes (old leaves), developing leaves from the six most upper apical nodes (young leaves), roots and fruits were collected and dried at 70°C for 24 h and the material ground to a find powder. Tomatoes were collected at the mature green/red ripe stage and were allowed one week of further maturation at the bench at room temperature (22 °C) before analysis. For the determination of soluble sugars, 100 mg of each sample was resuspended in 2 ml of water, sonicated and centrifuged for 10 min at 2,500 xg. Soluble sugar and proline contents were determined in the supernatant as described. Ion contents were determined by atomic absorption

spectrophotometry and chloride content by titration. Water content was calculated as (FW-DW)/FW, where FW and DW are the fresh and dry weight, respectively. Dry weight was obtained by placing the material at 70 °C until a constant weight was obtained. For the determination of soluble solid contents, the tomatoes were strained through a 20 μm mesh and Brix readings of the juice were obtained by refractrometry. Brix readings (oBrix) represent the concentrations of soluble solids as a percentage of total fresh weight.

Results and Discussion

[0084] A construct containing the Arabidopsis thaliana AtNHX1, coding for a vacuolar Na+/H+ antiport, was introduced into the genome of Lycopersicon esculentum cv Moneymaker. Forty-seven transgenic plants were obtained and six homozygous lines from these transgenic plants were obtained in the T2 generation (data not shown). Two of these homozygous lines were used in our experiments. These two lines were chosen because they grew more vigorously in high salinity. The overexpression of the vacuolar Na+/H+ antiport did not affect the growth of the transgenic plants (only one line of transgenic plants is shown) since similar growth was observed when the wild-type and the transgenic plants were grown in the presence of 5 mM NaCl (Figs 1A,B). Immunoblots of membrane fractions isolated from wild-type and transgenic plants only detected AtNHX1 in the tonoplast-enriched fractions from transgenic plants (Fig 1C), indicating the proper targeting of the Na+/H+ antiport to the vacuoles. In order to assess whether the enhanced expression of the vacuolar Na+/H+ antiport would allow plants to grow in high salt conditions, wild-type and transgenic plants were grown in the presence of 200 mM NaCl, a concentration that inhibits the growth of almost all crop plants. The growth of the wild-type plants was severely affected by the presence of 200 mM NaCl in the growth solution, plant growth was inhibited, most of the plants died or were severely stunted (Fig. 1D). On the other hand, the transgenic plants grew, flowered and produced fruit (Fig 1E).

[0085] To confirm that the presence of the Na+/H+ antiport protein resulted in increased Na+/H+ exchange, we monitored H+-dependent Na+ movements in tonoplast vesicles isolated from leaves. The vesicular lumen was acidified by the activation of the

vacuolar H+-PPIase in the presence of K+ ions, since the H+-PPIase activity is K+ dependent7. Once the pH gradient was established, the H+-pump activity was stopped by the addition of AMDP (amino-methylene-diphosphonate)8, NaCl was added and the rates of Na+/H+ exchange measured (Fig. 2A). Tonoplast vesicles isolated from transgenic plants displayed Na+/H+ exchange rates 7-fold higher than those from vesicles isolated from wild-type plants. Interestingly, K+/H+ exchange was also observed in the tonoplast vesicles after the addition of AMDP, in the absence of external Na+, (Fig. 2B) and the rates of K+/H+ exchange were significantly higher in vesicles isolated from the transgenic plants. These results indicate that the vacuolar Na+/H+ antiport was also able to mediate K+/H+ exchange, albeit with a lower specificity for K+ than for Na+. K+ ions are involved in a wide number of physiological processes and vacuolar pools generate the turgor needed to drive cell expansion9. Under K+ deficient growth conditions, vacuolar K+ concentrations decline while the cytosolic K+ concentrations remain relatively constant10. Cytosolic K+ concentrations decline only when the vacuolar K+ concentrations decrease to values around 20 mM11. The decrease in cytosolic K+ concentrations with the concomitant increase in cytosolic Na+/K+ ratio is the basis of cytosolic Na+ toxicity12. Given the cytosol-negative electrical potential difference at the tonoplast, an active K+ translocation mechanism into the vacuole has to be considered. Evidence of a K+/H+ antiport was found in tonoplast-enriched fractions from different plants6. Although the Arabidopsis sequencing project is completed, only putative K+/H+ antiports with similarity to the glutathione-regulated potassium-efflux system of E. coli13 have been sequenced (Accession numbers AAF78418, AAD10158, CCAB80872). Although their putative function has not yet been characterized in plants, in bacteria and yeast these transporters function as plasma membrane-bound potassium exchangers13,14. Although the role of vacuolar Na+/H+ antiports in glycophytes has yet to be established, its ubiquity in plants (Blumwald, in preparation) and its ability to mediate K+ transport would suggest that the vacuolar Na+/H+ antiport could also play a role in cellular K+ homeostasis.

[0086] We determined the ion, sugar, and proline contents of wild-type and transgenic plants grown at low (5 mM) NaCl and two independent transgenic lines grown at high (200 mM) NaCl (Fig. 3). It should be noted that a comparison with wild-type

plants grown at high salinity was not possible since all of the wild-type plants grown in these conditions were dead. At low salinity, no significant differences were seen in the content of Na+ (Fig. 3A), K+(Fig. 3B), Cl- (Fig. 3C) soluble sugars (Fig. 3D) or proline (Fig. 3D) of all tissues. Dramatic changes were seen in transgenic plants grown at high salinity. A 28- and 20-fold increase in Na+ content was seen in fully developed mature (old) and developing (young) leaves, respectively (Fig. 3A), and a similar increase in Cl- content was also observed (Fig. 3C). The K+ content of old leaves, young leaves and roots decreased a 5-, 2- and 4-fold, respectively (Fig. 3B). While no significant difference in soluble sugars was observed during growth in high salinity (Fig. 3D), a 3- and 5-fold increase in proline content was seen in leaves and fruits, respectively (Fig. 3E). The accumulation of proline in response to high salinity is well documented. Many prokaryotic and eukaryotic organisms accumulate proline during osmotic and salt stress15,16. Proline contributes to osmotic adjustment17, the protection of macromolecules during dehydration 18, and as a hydroxyl radical scavenger 19. Evidence supporting the role of proline during salt stress was obtained based on salt tolerance in transgenic tobacco plants with enhanced levels of proline biosynthesis20 and salt tolerance of Arabidopsis with suppressed levels of proline degradation21.

[0087] Taken together, our results demonstrate the ability of the transgenic plants to utilize salty water for growth. In spite of the high Na+ and Cl- content in the leaves of the transgenic plants grown at 200 mM NaCl, only a marginal increase in the Na+ and Cl- content of the fruits was observed. The K+ content of the leaves from transgenic plants grown in salt decreased while the K+ content of the transgenic fruits was higher than the K+ content of the fruits from plants grown at low salinity. These results clearly demonstrate that the enhanced accumulation of Na+, mediated by the vacuolar Na+/H+ antiport, allowed the transgenic plants to ameliorate the toxic effects of Na+ and the transgenic plants overcame salt-induced impaired nutrient acquisition7. Notably, transgenic plants grown in the presence of 200 mM NaCl produced fruits (Figs. 4A,B and Table 1). While the transgenic leaves accumulated Na+ to almost 1% of their dry weight, the fruits displayed only a marginal increase in Na+ content and a 25% increase in K+ content. The number of fruits per plant was similar, and although the fruits from the transgenic plants grown in 200 mM NaCl were somewhat smaller, no significant

difference was observed in their water content or total soluble solids content (Table 1). The low Na+ content of the transgenic fruits cannot be due to the lack of vacuolar Na+/H+ antiport since the protein was present in the fruit tissue (Fig. 4C). It has been demonstrated that in expanding fruit of many plant species, including tomato, more than 90% of the water transported into the fruit occurs through the phloem22,23,24. Thus the ability to maintain a high cytosolic K+/Na+ concentration ratio along the symplastic pathway was most probably responsible for the low Na+ content of the fruits.

[0088] Worldwide, more than 60 million hectares of irrigated land (representing 25% of the total irrigated acreage in the world) have been damaged by salt25. Our findings suggests the feasibility of producing salt tolerant transgenic plants that will produce edible crops.

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Table I. Plant and fruit yield of wild-type (WT) tomato plants grown in the presence of 5 mM NaCl and T2 transgenic plants overexpressing AtNHX1 (OEX1) grown in the presence of 5 mM and 200 mM NaCl. Plants were harvested 12 weeks after germination. Each value is the Mean ± SD (n = 10 individual plants).

	WT	OEX1	
	(5 mM NaCl)	(5 mM NaCl)	(200 mM NaCl)
Height (cm)	124.0±8.2	128.8±9.5	107.6±5.2
Fresh Weight (g) (without fruit)	1,270±103	1,329±110	1,123±134
Fruit per plant	17.2±1.3	17.8±.6	18.4±1.5
Fruit weight (g)	119.5±13.4	116.7±9.0	105.7±6.7
Fruit water content(%)	90.8±3.2	90.2±2.2	90.7±2.3
Solid solute content (^{o}Brix)	4.2±0.6	4.4±0.7	4.2±0.5

Table II.

SEQ ID No	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
2	NHX1 4324597	AAD16946	NHX1 Na+/H+ exchanger *Arabidopsis thaliana*	1 MLDSLVSKLP SLSTSDHASV VALNLFVALL CACIVLGHLL EENRWMNESI TALLIGLGTG 61 VTILLISKGK SSHLLVFSED LFFIYLLPPI IFNAGFQVKK KQFFRNFVTI MLFGAVGTII 121 SCTIISLGVT QFFKKLDIGT FDLGDYLAIG AIFAATDSVC TLQVLNQDET PLLYSLVFGE 181 GVVNDATSVV VFNAIQSFDL THLNHEAAFH LLGNFLYLFL LSTLLGAATG LISAYVIKKL 241 YFGRHSTDRE VALMMLMAYL SYMLAELFDL SGILTVFFCG IVMSHYTWHN VTESSRITTK 301 HTFATLSFLA ETFIFLYVGM DALDIDKWRS VSDTPGTSIA VSSILMGLVM VGRAAFVFPL 361 SFLSNLAKKN QSEKINFNMQ VVIWWSGLMR GAVSMALAYN KFTRAGHTDV RGNAIMITST 421 ITVCLFSTVV FGMLTKPLIS YLLPHQNATT SMLSDDNTPK SIHIPLLDQD SFIEPSGNHN 481 VPRPDSIRGF LTRPTRTVHY YWRQFDDSFM RPVFGGRGFV PFVPGSPTER NPPDLSKA
3	10716129	BAB16380	Na+/H+ exchanger *Ipomoea nil*	1 MAFGLSSLLQ NSDLFTSDHA SVVSMNLFVA LLCACIVLGH LLEENRWVNE SITALIIGLC 61 TGVVILLLSG GKSSHLLVFS EDLFFIYLLP PIIFNAGFQV KKKQFFVNFM TIMLFGAIGT 121 LISCSIISFG AVKIFKHLDI DFLDFGDYLA IGAIFAATDS VCTLQVLSQD ETPLLYSLVF 181 GEGVVNDATS VVLFNAIQSF DMTSFDPKIG LHFIGNFLYL FLSSTFLGVG IGLLCAYIIK 241 KLYFGRHSTD REVALMMLMS YLSYIMAELF YLSGILTVFF CGIVMSHYTW HNVTESSRVT 301 TRHSFATLSF VAETFIFLYV GMDALDIEKW KFVKNSQGLS VAVSSILVGL ILVGRAAFVF 361 PLSFLSNLAK KNSSDKISFR QQIIIWWAGL MRGAVSIALA YNKFTTSGHT SLHENAIMIT 421 STVTVVLFST VVFGLMTKPL INLLLPPHKQ MPSGHSSMTT SEPSSPKHFT VPLLDNQPDS 481 ESDMITGPEV ARPTALRMLL RTPTHTVHRY WRKFDDSFMR PVFGGRGFVP FVAGSPVEQS 541 PR
4	14039961	AAK53432	Na+/H+ Antiporter *Suaeda maritima* subsp. salsa	1 MLSQLSSFFA SKMDMVSTSD HASVVSMNLF VALLRGCIVI GHLLEENRWM NESITALLIG 61 LSTGIIILLI SGGKSSHLLV FSEDLFFIYL LPPIIFNAGF QVKKKQFFRN FITIILFGAV 121 GTLVSFIIIS LGSIAIFQKM DIGSLELGDL LAIGAIFAAT DSVCTLQVLN QDETPLLYSL 181 VFGEGVVNDA TSVVLFNAIQ NFDLTHIDHR IAFQFGGNFL YLFFASTLLG AVTGLLSAYV 241 IKKLYFGRHS TDREVALMML MAYLSYMLAE LFYLSGILTV FFCGIVMSHY TWHNVTESSR 301 VTTKHAFATL SFVAEIFIFL YVGMDALDIE KWRFVSDSPG TSVAVSSILL GLHMVGRAAF 361 VFPFAFLMNL SKKSNSEKVT FNQQIVIWWA GLMKSAVSVA LAYNQFSRSG HTQLRGNAIM 421 ITSTITVVLF STMVFGLLTK PLILFMLPQP KHFTSASTVS DLGSPKSFSL PLLEDRQDSE 481 ADLGNDDEEA YPRGTIARPT SLRMLLNAPT HTVHHYWRRF DDYFMRPVFG GRGFVPFVPG 541 SPTEQSITNF VTENIS

Seq ID No	Protein Number (GI)	Protein Accession	Protein Description (species)	Sequence
5	14211574	BAB56105	Na+/H+ Antiporter *Petunia* x *hybrida*	1 MAFDFGTLLG NVDRLSTSDH QSVVSINLFV ALICACIVIG HLLEENRWMN ESITALVIGS 61 CTGIVILLIS GGKNSHILVF SEDLFFIYLL PPIIFNAGFQ VKKKSFFRNF STIMLFGALG 121 TLISFIIISL GAIGIFKKMN IGSLEIGDYL AIGAIFSATD SVCTLQVLNQ DETPLLYSLV 181 FGEGVVNDAT SVVLFNAIQN FDLSHIDTGK AMELVGNFLY LFASSTALGV AAGLLSAYII 241 KKLYFGRHST DREVAIMILM AYLSYMLAEL FYLSAILTVF FSGIVMSHYT WHNVTESSRV 301 TTKHTFATLS FIAEIFIFLY VGMDALDIEK WKFVSDSPGI SVQVSSILLG LVLVGRAAFV 361 FPLSFLSNLT KKTPEAKISF NQQVTIWWAG LMRGAVSMAL AYNQFTRGGH TQLRANAIMI 421 TSTITVVLFS TVVFGLMTKP LIRILLPSHK HLSRMISSEP TTPKSFIVPL LDSTQDSEAD 481 LERHVPRPHS LRMLLSTPSH TVHYYWRKFD NAFMRPVFGG RGFVPFAPGS PTDPVGGNLQ
6	14211578	BAB56107	Na+/H+ Antiporter *Torenia hybrida*	1 MGFESVIKLA ASETDNLWSS GHGSVVAITL FVTLLCTCIV IGHLLEENRW MNESIIALII 61 GLATGVIILL ISGGKSSHLL VFSEDLFFIY ALPPIIFNAG FQVKKKSFFR NFATIMMFGA 121 VGTLISFIII SLGTIAFFPK MNMRLGVGDY LAIGAIFAAT DSVCTLQVLS QDETPLLYSL 181 VFGEGVVNDA TSVVLFNAVQ NFDLPHMSTA KAFELVGNFF YLFATSTVLG VLTGLLSAYI 241 IKKLYFGRHS TDREVAIMIL MAYLSYMLAE LFDLSGILTV FFCGIVMSHY TWHNVTENSR 301 VTTKHTFATL SFVAEIFIFL YVGMDALDIE KWRFVSGSMT TSAAVSATLL GLVLLSRAAF 361 VFPLSFLSNL AKKSPLEKIS LRQQIIIWWA GLMRGAVSMA LAYKQFTREG LTVERENAIF 421 ITSTITIVLF STVVFGLMTK PLINLLIPSP KLNRSVSSEP LTPNSITIPL LGESQDSVAE 481 LFSIRGQTSQ GGEPVARPSS LRMLLTKPTH TVHYYWRKFD NAFMRPVFGG RGFVPYVPGS 541 PTERSVRNWE EETKQ
7	14488270	BAB60901	Na+/H+ exchanger *Ipomoea tricolor*	1 MAFGLSSLLQ NSELFTSDHA SVVSMNLFVA LLCACIVLGH LLEENRWVNE SITALIIGLC 61 TGVVILLLSR GKSSHLLVFS EDLFFIYLLP PIIFNAGFQV KKKQFFVNFM TIMLFGAIGT 121 LISCSIISFG AVKIFKHLDI DFLDFGDYLA IGAIFAATDS VCTLQVLSQD ETPLLYSLVF 181 GEGVVNDATS VVLFNAIQSF DMTSFDPKIG LHFIGNFLYL FLSSTFLGVG IGLLCAYIIK 241 KLYFGRHSTD REVALMMLMS YLSYIMAELF YLSGILTVFF CGIVMSHYTW HNVTESSRVT 301 TRHSFATLSF VAETFIFLYV GMDALDIEKW KFVKNSQGLS VAVSSILVGL ILVGRAAFVF 361 PLSFLSNLAK KNSSDKISFR QQIIIWWAGL MRGAVSIALA YNKFTTSGHT SLHENAIMIT 421 STVTVVLFST VVFGLMTKPL INLLLPPHKQ IASGHSSMTT SEPSSPKHFA VPLLDNQHDS 481 ESDMITGPEV ARPTALRMLL RTPTHTVHRY WRKFDDSFMR PVFGGRGFVP FVAGSPAEQS 541 PR

SEQ ID No	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
8	4585981	AAD25617	similar to Na+/H+-exchanging proteins *Arabidopsis thaliana*	1 MISPVEHDPQ GQVKQQQAAG VGILLQIMML VLSFVLGHVL RRHRFHYLPE ASGSLLIGLI 61 VGILANISDT ETSIRFCPPP SIPEFSLLSF PRSLVCSFYS VSGRGLISTK SSSSCFCCLP 121 SYYILCFNIC ISSFKFAAAM LCIMDVIFLD IIHLFEPSQV SVFNLNHSFL TLEPLLPLLS 181 SELLSLQLLL VVCYLGGSMY LMYKLPFVEC LMFGALISAT DPVTVLSIFQ VLLLFLLLSV 241 STGYKYSHDV GTDVNLYALV FGESVLNDAV SFYYLLRYWA LPFKTMSLVN RQSSSGEHFF 301 MVVIRFFETF AGSMSAGLAI SFLNSFYTVV FTLLILSEHI VNVMSLFSLF STSIHACRRC 361 WSLRHCFYTL HRNCNRRVMK RYTFSNLSEA SQSFVSSFFH LISSLAETFT FIYMGFDIAM 421 EQHSWSHVGA VNVFGCAYLV NLFRQENQKI PMKHQKALWY SGLRGAMAFA LALQSLHDLP 481 EGHGQIIFTA TTTIVVVTVT FVLLIGGSTG KMLEALEVVG DDLDDSMSEV NSRRSTLISL 541 NIGASSDEDT SSSGSRFKMK LKEFHKTGDG DGDGE
9	8515714	AAF76139	putative Na+/H+ antiporter SOS1 *Arabidopsis thaliana*	1 MTTVIDATMA YRFLEEATDS SSSSSSSKLE SSPVDAVLFV GMSLVLGIAS RHLLRGTRVP 61 YTVALLVIGI ALGSLEYGAK HNLGKIGHGI RIWNEIDPEL LLAVFLPALL FESSFSMEVH 121 QIKRCLGQMV LLAVPGVLIS TACLGSLVKV TFPYEWDWKT SLLLGGLLSA TDPVAVVALL 181 KELGASKKLS TIIEGESLMN DGTAIVVFQL FLKMAMGQNS DWSSIIKFLL KVALGAVGIG 241 LAFGIASVIW LKFIFNDTVI EITLTIAVSY FAYYTAQEWA GASGVLTVMT LGMFYAAFAR 301 TAFKGDSQKS LHHFWEMVAY IANTLIFILS GVVIAEGILD SDKIAYQGNS WRFLFLLYVY 361 IQLSRVVVVG VLYPLLCRFG YGLDWKESII LVWSGLRGAV ALALSLSVKQ SSGNSHISKE 421 TGTLFLFFTG GIVFLTLIVN GSTTQFVLRL LRMDILPAPK KRILEYTKYE MLNKALRAFQ 481 DLGDDEELGP ADWPTVESYI SSLKGSEGEL VHHPHNGSKI GSLDPKSLKD IRMRFLNGVQ 541 ATYWEMLDEG RISEVTANIL MQSVDEALDQ VSTTLCDWRG LKPHVNFPNY YNFLHSKVVP 601 RKLVTYFAVE RLESACYISA AFLRAHTIAR QQLYDFLGES NIGSIVINES EKEGEEAKKF 661 LEKVRSSFPQ VLRVVKTKQV TYSVLNHLLG YIENLEKVGL LEEKEIAHLH DAVQTGLKKL 721 LRNPPIVKLP KLSDMITSHP LSVALPPAFC EPLKHSKKEP MKLRGVTLYK EGSKPTGVWL 781 IFDGIVKWKS KILSNNHSLH PTFSHGSTLG LYEVLTGKPY LCDLITDSMV LCFFIDSEKI 841 LSLQSDSTID DFLWQESALV LLKLLRPQIF ESVAMQELRA LVSTESSKLT TYVTGESIEI 901 DCNSIGLLLE GFVKPVGIKE ELISSPAALS PSNGNQSFHN SSEASGIMRV SFSQQATQYI 961 VETRARAIIF NIGAFGADRT LHRRPSSLTP PRSSSSDQLQ RSFRKEHRGL MSWPENIYAK 1021 QQQEINKTTL SLSERAMQLS IFGSMVNVYR RSVSFGGIYN NKLQDNLLYK KLPLNPAQGL 1081 VSAKSESSIV TKKQLETRKH ACQLPLKGES STRQNTMVES SDEEDEDEGI VVRIDSPSKI 1141 VFRNDL

SEQ ID No	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
10	9857314	BAB11940	Na/H antiporter Nhx1 *Atriplex gmelini*	1 MWSQLSSLLS GKMDALTTSD HASVVSMNLF VALLCGCIVI GHLLEENRWM NESITALLIG **61 LATGVVILLI SGGKSSHLLV FSEDLFFIYL LPPIIFNAGF** QVKKKQFFRN FITIVLF**GAV** **121 GTL**VSFTIIS LGALSIFKKL DIGTLELADY LAI**GAIFAAT** DSVCTLQVLN QDETP**LLYSL** **181 VFGEGVVNDA TSVVLFNA**IQ SFDLTRIDHR IALQFMGNFL YLFIASTILG **AFTGL**LSAYI 241 IKKLYFGRHS TDREVALMML MAYLSYMLAE LFYLSGILTV **FFCGIVMSHY** TWHNVTESSR 301 VTTK**HAFATL SFVAEVFLFL YVGMDAL**DIE KWRFVSDSPG **ISVAVSSILL GLVMVGRAAF** **361 VFPL**SWLMNF AKKSQSEKVT FNQQIVIWWA **GLMRGA**VSMA LAYNQFTRSG HTQLRGN**AIM** **421 ITSTISVVLF STMVFGLLTK** PLIMFLLPQP KHFTSCSTVS DVGSPKSYSL PLLEGNQDYE 481 VDVGNGNHED TTEPRTIVRP SSLRMLLNAP THTVHHYWRK FDDSFMRPVF GGRGFVPFVP 541 GSPTEQSTNN LVDRT
11	NHA1 6323167	NP_013239	Putative Na+/H+ antiporter; Nha1p *Saccharomyces cerevisiae*	1 MAIWEQLEVS KAHVAYACV**G VFSSIFSLVS** LYVKEKL**YIG ESTVAGIFGL IVGPVCLNWF** 61 NPLKW**GNSDS ITLEITRIVL** CLQIFAVAVE LPRKYMLKHW VSVTMLLLPV MTAGWLIIGL 121 FVWILIPGL**N FSASLLISAC** ITATDPILAQ SVVSGKFAQR VPGHLRNLLS AESG**CNDGMA** 181 FPFLFLSMNL ILHPGNGREI VKDWICVTIL YECLF**GCLLG CFIGYVGRIT** IRFAEKKNII 241 DRESFLAFYV VLAFMCAGF**G SILGVDDLLV** SFAA**GATFAW** DGWFSQKTQE SNVSTVIDL**L** **301 LNYAYFIYFG AIIPWSQFN**N GEIGTNVWR**L IILSIVVIFL RRIPAVMILR** PLIPDIKSWR 361 EALFVGHFGP IGV**GAIFAAI** LARGELESTF SDEPTPLNVV PSKEESKHWQ LIACIWPITC 421 FFIVTSIIVH **GSSVAII**TLG RHLNTITLTK TFTTHTTNGD NGKSSWMQRL PSLDKAGRSF 481 SLHRMDTQMT LSGDEGAEEE **GGGRKGL**AGG EDEEGLNNDQ **IGSVATS**GIP ARPAGGMPRR 541 RKLSRKEKRL NRRQKLRNKG REIFSSRSKN EMYDDDELND LGRERLQKEK EARAATFALS 601 TAVNTQRNEE IGMGGDEEED EYTPEKEYSD NYNNTPSFES SERSSSLRGR TYVPRNRYDG 661 EETESEIESE DEMENESERS MASSEERRIR KMKEEEMKPG TAYLDGNRMI IENKQGEILN 721 QVDIEDRNEA RDDEVSVDST AHSSLTTTMT NLSSSSGGRL KRILTPTSLG KIHSLVDKGK 781 DKNKNSKYHA FKIDNLLIIE NEDGDVIKRY KINPHKSDDD KSKNRPRNDS VVSRALTAVG 841 LKSKANSGVP PPVDEEKAIE GPSRKGPGML KKRTLTPAPP **RGVQDSL**DLE DEPSSEEDLG 901 DSYNMDDSED YDDNAYESET EFERQRRLNA LGEMTAPADQ DDEELPPLPV EAQTGNDGPG 961 TAEGKKKQKS AAVKSALSKT LGLNK

SEQ ID NO	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
12	NHX1 6320663	NP_010744	Required for intracellular sequestration of Na+; Nhx1p *Saccharomyces cerevisiae*	1 MLSKVLLNIA FKVLLTTAKR AVDPDDDDEL LPSPDLPGSD DPIAGDPDVD LNPVTEEMFS 61 SWALFIMLLL LISALWSSYY LTQKRIRAVH ETVLSIFYGM VIGLIIRMSP GHYIQDTVTF 121 NSSYFFNVLL PPIILNSGYE LNQVNFFNNM LSILIFAIPG TFISAVVIGI ILYIWTFLGL 181 ESIDISFADA MSVGATLSAT DPVTILSIFN AYKVDPKLYT IIFGESLLND AISIVMFETC 241 QKFHGQPATF SSVFEGAGLF LMTFSVSLLI GVLIGILVAL LLKHTHIRRY PQIESCLILL 301 IAYESYFFSN GCHMSGIVSL LFCGITLKHY AYYNMSRRSQ ITIKYIFQLL ARLSENFIFI 361 YLGLELFTEV ELVYKPLLII VAAISICVAR WCAVFPLSQF VNWIYRVKTI RSMSGITGEN 421 ISVPDEIPYN YQMMTFWAGL RGAVGVALAL GIQGEYKFTL LATVLVVVVL TVIIFGGTTA 481 GMLEVLNIKT GCISEEDTSD DEFDIEAPRA INLLNGSSIQ TDLGPYSDNN SPDISIDQFA 541 VSSNKNLPNN ISTTGGNTFG GLNETENTSP NPARSSMDKR NLRDKLGTIF NSDSQWFQNF 601 DEQVLKPVFL DNVSPSLQDS ATQSPADFSS QNH
13	NHX2 15229877	NP_187154	NHX2 Na+/H+ exchanger *Arabidopsis thaliana*	1 MTMFASLTSK MLSVSTSDHA SVVSLNLFVA LLCACIVIGH LLEENRWMNE SITALLIGLG 61 TGVVILLISR GKNSHLLVFS EDLFFIYLLP PIIFNAGFQV KKKQFFRNFV TIMAFGAIGT 121 VVSCTIISLG AIQFFKKLDI GTFDLGDFLA IGAIFAATDS VCTLQVLNQD ETPLLYSLVF 181 GEGVVNDATS VVLFNAIQSF DLTHLNHEAA FQFLGNFFYL FLLSTGLGVA TGLISAYVIK 241 KLYFGRHSTD REVALMMLMA YLSYMLAELF ALSGILTVFF CGIVMSHYTW HNVTESSRIT 301 TKHAFATLSF LAETFIFLYV GMDALDIEKW RFVSDSPGTS VAVSSILMGL VMLGRAAFVF 361 PLSFLSNLAK KHQSEKISIK QQVVIWWAGL MRGAVSMALA YNKFTRSGHT ELRGNAIMIT 421 STITVCLFST MVFGMLTKPL IRYLMPHQKA TTSTTSMLSD DSTPKSIHIP LLDGEQLDSF 481 ELPGSHQDVP RPNSLRGFLM RPTRTVHYYW RQFDDAFMRP VFGGRGFVPF VPGSPTERSS 541 HDLSKP
14	NHX3 15240159	NP_200358	NHX3 Na+/H+ exchanger *Arabidopsis thaliana*	1 MSIGLTEFVT NKLAAEHPQV IPISVFIAIL CLCLVIGHLL EENRWVNESI TAILVGAASG 61 TVILLISKGK SSHILVFDEE LFFIYLLPPI IFNAGFQVKK KKFFHNFLTI MSFGVIGVFI 121 STVIISFGTW WLFPKLGFKG LSARDYLAIG TIFSSTDTVC TLQILHQDET PLLYSLVFGE 181 GVVNDATSVV LFNAVQKIQF ESLTGWTALQ VFGNFLYLFS TSTLLGIGVG LITSFVLKTL 241 YFGRHSTTRE LAIMVLMAYL SYMLAELFSL SGILTVFFCG VLMSHYASYN VTESSRITSR 301 HVFAMLSFIA ETFIFLYVGT DALDFTKWKT SSLSFGGTLG VSGVITALVL LGRAAFVFPL 361 SVLTNFMNRH TERNESITFK HQVIIWWAGL MRGAVSIALA FKQFTYSGVT LDPVNAAMVT 421 NTTIVVLFTT LVFGFLTKPL VNYLLPQDAS HNTGNRGKRT EPGSPKEDAT LPLLSFDESA 481 STNFNRAKDS ISLLMEQPVY TIHRYWRKFD DTYMRPIFGG PRRENQPEC

SEQ ID NO	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
15	NHX4 15230706	NP_187288	NHX4 Na+/H+ exchanger *Arabidopsis thaliana*	1 MVIGLSTMLE KTEALFASDH ASVVSMNLFV ALLCACIVLG HLLEETRWMN ESITALI**IGS** **61** **CTGI**VILLIS **GGKSSRILVF** **SEDLFFIYLL** **PPIIFNAGFQ** VKKKQFFRNF MTIMLF**GAIG** **121** **TL**ISFVIISF GAKHLFEKMN IGDLTIADYL AI**GAIFSATD** SVCTLQVLNQ DETP**LLYSLV** **181** **FGEGVVNDAT** SVVLFNAIQR FDLTNINSAI ALEFAGNFFY LFILSTAL**GV** **AAGL**LSAFVI 241 KKLYIGRHST DREVALMMLL AYLSYMLAEL FHLSSILTVF FC**GIVMSH**YT WHNVTDKSKV 301 TTKHT**FAAMS** **FLAEIFIFLY** **VGMDAL**DIEK WDVVRNSPGQ **SIGVSSILLG** **LILLGRAAFV** **361** **FPL**SFLSNLT KSSPDEKIDL KKQVTIWWA**G** **LMRGA**VSMAL AYNQFTTSGH TKVLGNAIMI 421 TSTITVVLFS TVVFGLLTKP LVKHLQPSSK QSSTTALQIT LRSSFHDPIL HEPLLSTQGQ 481 SEYDPEQHVS FRMFWKSPSR AIHHYWRKFD NAVMRRIFGG RGVSPVVPGS PIENSVPQWS 541 EEVENKEQNG EP
16	NHX5 30695721	NP_175839	NHX5 Na+/H+ exchanger *Arabidopsis thaliana*	1 MEEVMISPVE HDPQGQVKQQ QAAGVGILLQ IMMLVLSFVL GHVLRRHRFH YLPEAS**GLIV** **61** **GIL**ANISDTE TSIRFCPPPS IPEFSLLSFP RSLKPFFSNF **GAIVTFA**IIG **TFVAS**VVTGG 121 LVYLGGSMYL MYKLPFVECL MF**GALISATD** PVTVLSIFQD VGTD**VNLYAL** **VFGESVLNDA** **181** **VSFYYLL**RYW ALPFKFFETF **AGSMSAE**HLF KYAGLDTENL QNLECCLFVL FPYFSYMLAE 241 **GVGLSG**IVSI LFTGIVMKRY TFSNLSEASQ SFVSSFFHLI SSLAETFTFI YMGFDIAMEQ 301 HSWSHVGFIL FSIVSSFTDR QAVNVFGCAY LVNLFRQENQ KIPMKHQKAL WYS**GLRGAMA** 361 FALALQSLHD LPEGHGQIIF TATTTIVVVT VLLIGGSTGK MLEALEVVGD DLDDSMSEGF 421 EESDHQYVPP PFSIGASSDE DTSSSGSRFK MKLKEFHKTT TSFTALDKNF LTPFFTTNSG 481 DGDGDGE
17	NHX6 22330742	NP_178079	NHX6 Na+/H+ exchanger *Arabidopsis thaliana*	1 MSSELQISPA IHDPQGQEKQ QQAAGVGILL QIMMLVLSFV LGHVLRRHKF YYLPEASASL 61 **LIGLIVGGLA** NISNTETSIR FVELFLISFF RHG**SISTMSS** **SFCFCCLPSY** **YIL**KIEYLGG 121 VMFLMYRLPF VECLM**FGSLI** **SATD**PVTVLS IFQEL**GSDVN** **L**YALVFGESV LNDADEIVT**L** **181** **LIRSFSFLCC** **FWQMAISLYR** **TM**SLVRSHSS GQNFFMVIVR FLETFVGS**MS** **AAMKYFILMY** **241** **SLLLSVY**RTW SAVSSYFFHI SRNK**TLLFYT** **SYVSIYFTLI** **EIVQFVM**KHY TYSNLSANSQ 301 **RFVSAFFHLI** **SSLAETFVFI** **YMGF**DIAMEK HSWAANVFGC GYLVNLARPA HRKIPMTHQK 361 **ALWYSGKILL** **CVPLSSYCFY** **SSV**INTKICG FCI**GLRGAMA** FALALQSVHD LPEGHGQTIF 421 TATTAIVVLT VLLIGGSTGT MLEALEVVGD SHDTSLGDGF EVVNSRYMTS YDDEDTPPGS 481 GFRTKLREFH KSAASFTELD RNYLTPFFTS NNGDYDDEGN MEQHHGNNII L

SEQ ID No	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
18	NHX7 22325422	NP_178307	NHX7 Na+/H+ exchanger *Arabidopsis thaliana*	1 MTSIIGAALP YKSPEKAIAS SSYSAENDSS PVDAVIF**AGT** **SLVLGTAC**RY LFNGTRVPYT 61 VVLLV**IGIFL** **GSLEYGTKHN** **L**GKLGHGIRI WN**GINPDLLL** **AVFLPVLLFE** **SSF**SMDVHQI 121 KRCMGQMVLL AGP**GVLISTF** CLGALIKLTF PYNWDWKTSL **LLGGLLGA**TD PVAVVALLKE 181 LGASKKMTTL IDGESLMNDG VSVVVFQLFF KMVMGHNSDW GSIIKFLVQN SFGAV**GIGLA** **241** **F**GIASVFWLK FIFNDTVAQI TVTLSASYFA YYTAQEWAGV SGILTVMIL**G** **MFFAA**FARTA 301 FKGDSHQSLH HFWYFTTQEM AAYIANTLVF MLS**GVIIAES** VLS**GQTISYK** AIKWKFISQF 361 RYGNK**AVLQF** **LFLTGGIVFL** **TLVVNGST**TQ LLLHLLRMDT LTATKKRILE YTKFEMMKTA 421 LKAFENLGDD EELGSADWPT VIRHISSLKD LEGRQVNPHD GYEAGSLDPT NIMDIRVQAA 481 YWEMLDDGRI TQCTANVLMQ SVDEALDLVS TSSLSDWRGL EPRVHFPNYY KFLQSKIIPH 541 KLVTHLIVER LESACYISSA FLRAHRIARQ QLHIFLGNSN IASTVINESE VEGEEAKQFL 601 EDVRDSFPQV LSVLKTRQVT HYVLNHLNGY IKNLEKVGLL EGKEVSHLHD VVQSDLKKLL 661 RHPPSLKLPN VDDLITSNPL LKDRSSFRSL AIGETDA
19	NHX8 15223849	NP_172918	NHX8 Na+/H+ exchanger *Arabidopsis thaliana*	1 MTSIIGAALP YKSPEKAIAS SSYSAENDSS PVDAVIF**AGT** **SLVLGTAC**RY LFNGTRVPYT 61 VVLLV**IGIFL** **GSLEYGTKHN** **L**GKLGHGIRI WN**GINPDLLL** **AVFLPVLLFE** **SSF**SMDVHQI 121 KRCMGQMVLL AGP**GVLISTF** CLGALIKLTF PYNWDWKTSL **LLGGLLGA**TD PVAVVALLKE 181 LGASKKMTTL IDGESLMNDG VSVVVFQLFF KMVMGHNSDW GSIIKFLVQN SFGAV**GIGLA** **241** **F**GIASVFWLK FIFNDTVAQI TVTLSASYFA YYTAQEWAGV SGILTVMIL**G** **MFFAA**FARTA 301 FKGDSHQSLH HFWYFTTQEM AAYIANTLVF MLS**GVIIAES** VLS**GQTISYK** AIKWKFISQF 361 RYGNK**AVLQF** **LFLTGGIVFL** **TLVVNGST**TQ LLLHLLRMDT LTATKKRILE YTKFEMMKTA 421 LKAFENLGDD EELGSADWPT VIRHISSLKD LEGRQVNPHD GYEAGSLDPT NIMDIRVQAA 481 YWEMLDDGRI TQCTANVLMQ SVDEALDLVS TSSLSDWRGL EPRVHFPNYY KFLQSKIIPH 541 KLVTHLIVER LESACYISSA FLRAHRIARQ QLHIFLGNSN IASTVINESE VEGEEAKQFL 601 EDVRDSFPQV LSVLKTRQVT HYVLNHLNGY IKNLEKVGLL EGKEVSHLHD VVQSDLKKLL 661 RHPPSLKLPN VDDLITSNPL LKDRSSFRSL AIGETDA
20	15982204	CAC84522	Na+/H+ antiporter, isoform 1 *Lycopersicon esculentum*	1 MGLDAVARLG VSILSDGDQV SVDSITLFVA LLCGCIVIGH LLEESRWIND SITTLVIGLS 61 TGGIILLTTK GKSSHLLEFD EQLFFIYVLP PIIFNAGFQV KKKQFFRNFV TIMLFGAVGT 121 LISFSIISFG AKELLGKLDI GFLELRDYLA IGAIFSATDS VCTLQALNQD ETPRLYSLVF 181 GEGVVNDATS VVLFNAIQKL DLSHINSRAA LVFTGNFLYL FLASTFLGVL IGLLSAYLIK 241 KIYLGRHSTD REVALMILMA YLSYVMAELF DLSGILTVFI CGIVMSHYTW HNVTFNSKVT 301 TRHAFATLSF IAEIFIFLYV GMDALDIEKW RFVKDSPGKS VGVSAALLGL VLVGRACFVF 361 PLSLFSNCLK RSEHDKFGLK LQVTIWWAGL MRGSVSMALA YNQFTRFGHT QQPGNAVMIT 421 STITIVLFST VVFGLITKPL VRFLLPSSQG FNNLISSEQS FARPLLTNEQ ELELEMGNVD 481 PVRPSGLSIL LKEPSYTIHN HWRRFDDAFM RPLFGGRGFV PDAPELSKGG CDQY

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21	15982206	CAC83608	Na+/H+ antiporter, isoform 2 *Lycopersicon esculentum*	1 MEDHLQISPA GAKAIPGKEQ QAAGYGILLQ IMMLVLSFVI GHVLRRRHFY YIPEASASLL 61 IGLIVGGLAN VSDTETSIRA WFNFHEEFFF LFLLPPIIFQ SGFSLSPKPF FSNFGAIITF 121 AILGTFIASF VTGILVYLGG VTYLMYRLPF VECLMFGALI SATDPVTVLS IFQELGTDVN 181 LYALVFGESV LNDAMAISLY RTMSLVRSHM STDQNYFMIT IRFVETFMGS LSAGVGVGFV 241 SALLFKYAGL DIDNLQNLES CLFVLFPYFS YMLAEGLGLS GIVSILFTGV VMKRYTYPNL 301 SESSQRFVSA FFHLISSLAE TFVFIYMGFD IAMEKHSWSH VGFIFFSILF IVIARAANVF 361 GCAYLVNLVR PPHQKIPAKH QKALWYSGLR GAMAFALALQ PVHDLPEGHG QAIFTATTAI 421 VVLTVLIIGG SAGTMLEALE VVGDGQSGSM DETFEGNNGY IAPSYRDESY DGEPSSGNRF 481 RMKLKEFHKS TTSFSALDKN YLTPFFTTQG GDEDEDEPIM HSSRRAGYDG H
22	5731737	BAA83337	OsNHX1 *Oryza sativa* (japonica cultivar-group)	1 MGMEVAAARL GALYTTSDYA SVVSINLFVA LLCACIVLGH LLEENRWVNE SITALIIGLC 61 TGVVILLMTK GKSSHLFVFS EDLFFIYLLP PIIFNAGFQV KKKQFFRNFM TITLFGAVGT 121 MISFFTISIA AIAIFSRMNI GTLDVGDFLA IGAIFSATDS VCTLQVLNQD ETPFLYSLVF 181 GEGVVNDATS IVLFNALQNF DLVHIDAAVV LKFLGNFFYL FLSSTFLGVF AGLLSAYIIK 241 KLYIGRHSTD REVALMMLMA YLSYMLAELL DLSGILTVFF CGIVMSHYTW HNVTESSRVT 301 TKHAFATLSF IAETFLFLYV GMDALDIEKW EFASDRPGKS IGISSILLGL VLIGRAAFVF 361 PLSFLSNLTK KAPNEKITWR QQVVIWWAGL MRGAVSIALA YNKFTRSGHT QLHGNAIMIT 421 STITVVLFST MVFGMMTKPL IRLLLPASGH PVTSEPSSPK SLHSPLLTSM QGSDLESTTN 481 IVRPSSLRML LTKPTHTVHY YWRKFDDALM RPMFGGRGFV PFSPGSPTEQ SHGGR
23	14211576	BAB56106	Na+/H+ antiporter, *Nierembergia caerulea*	1 MAFDFGTLLG KMNNLTTSDH QSVVSVNLFV ALICACIVIG HLLEENRWMN ESITALVIGS 61 CTGVIILLIS GGKNSHILVF SEDLFFIYLL PPIIFNAGFQ VKKKSFFRNF STIMLFGAVG 121 TLISFIIISA GAIGIFKKMD IGHLEIGDYL AIGAIFAATD SVCTLQVLNQ EETPLLYSLV 181 FGEGVVNDAT SVVLFNAVQN FDLSHISTGK ALQLIGNFLY LFASSTFLGV AVGLLSAFII 241 KKLYFGRHST DREVAIMILM AYLSYMLAEL FYLSGILTVF FCGIVMSHYT WHNVTESSRV 301 TTKHTFATLS FIAEIFIFLY VGMDALDIEK WKFVSDSPGT SIKVSSILLG LVLVGRGAFV 361 FPLSFLSNLT KKNPEDKISF NQQVTIWWAG LMRGAVSMAL AYNQFTRGGH TQLRANAIMI 421 TSTITVVLFS TVVFGLMTKP LILLLLPSQK HLIRMISSEP MTPKSFIVPL LDSTQDSEAD 481 LGRHVPRPHS LRMLLSTPSH TVHYYWRKFD NAFMRPVFGG RGFVPFVPGS PTEPVEPTEP 541 RPAESRPTEP TDE

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24	15812035	AAK27314	Na+/H+ exchanger *Citrus* x *paradisi*	1 MDQAISSVVR KLQMVNTSDH NSVVSINIFV ALPCASIVIG HLLEESRWMN ESITALLIGV 61 CAGVIILLTT GGKSSHLFVF SEDLFFIYVL PPIIFNAGFQ VKKKQFFRNF ITIMLFGAIG 121 TLVSCTIISL GVIQFFKKLD IGTLDIGDYL AIGAIFAATD SVCTLQVLNQ DDTPLLYSLV 181 FGEGVVNDAT SVVLFNAIQS FDLTHINTRS AFQFIGNFLY LFFTSTLLGV IGGLLSAYVI 241 KKLYFGRHST DREVAIMVLM AYLSYMLAEL FYLSGILTVF FCGIVMSHYT WHNVTESSRV 301 TTKHTFATLS FVAEIFTFLY VGMDALDIEK WRFVKGSPGT SVAASAMLMG LIMAGRAAFV 361 FPLSFLTNLA KKSPTEKISI KQQVIIWWAG LMRGAVSMAL AYNQFTRSGH TQLRENAIMI 421 TSTITVVLFS TVVFGLMTEP LIRLLLPHPK HTTNHILSDP STPKSLSQPL LEEGQQDSYA 481 DLVGPTVPRP GSLRALLTTP THTVHYYWRK FDDAFMRPVF GGRGFAPFVP GSPTERSVRG 541 GQ
25	15027833	AAK76737	Na+/H+ antiporter *Triticum aestivum*	1 MGLDLGALAL KYTGLAVSDH DSIVAINIFI ALLCGCIVFG HLLEGNRWVN ESTTALVLGL 61 ITGGVILICT KGVNSRILIF SEDIFFIYLL PPIIFNAGFQ VKKKQFFRNF ATIILFGAAG 121 TLISFVIITF GAMGLFSKLD VGPLELGDYL AIGAIFSATD SVCTLQVLNQ DEAPLLYSLV 181 FGEGVVNDAT SVVLFNAIQN IDINHFDVFV LLQFIGKFLY LFFTSTVLGV AAGLLSAYII 241 KKLCFARHST DREVAIMILM AYLSYMLSML LDLSGILTVF FCGIVMSHYT WHNVTESSRV 301 TTKHTFATLS FIAEIFLFLY VGMDALDIDK WKLASSSPKK PIALSAVILG LVMVGRAAFV 361 FPLSFLSNLS KKESHPKISF NQQVIIWWAG LMRGAVSIAL AYNKFTTSGH TAVRVNAVMI 421 TSTIIVVLFS TMVFGLLTKP LINLLIPPRP GTAADISSQS FLDPLTASLL GSDFDVGQLT 481 PQTNLQYLLT MPTRSVHRVW RKFDDKFMRP MFGGRGFVPF VPGSPIERSV HGPGLLGTVT 541 EAEDRS
26	28575021	AAK76738	Na+/H+ antiporter *Triticum aestivum*	1 MGYQVVAAQL ARLSGALGTS DHASVVSITL FVALLCACIV LGHLLEENRW LNESITALII 61 GLCTGVVILM TTKGKSSHVL VFSEDLFFIY LLPPIIFNAG FQVKKKQFFR NFMAITLFGA 121 VGTMMSFFTI SLAAIAIFSR MNIGTLDVSD FLAIGAIFSA TDSVCTLQVL NQDETPFLYS 181 LVFGEGVVND ATSVVLFNAL QNFDPNQIDA IVILKFLGNF CYLFVSSTFL GVFTGLLSAY 241 VIKKLYIGRH STDREVALVM LMAYLSYMLA ELLDLSGILT VFFCGIVMSH YTWHNVTESS 301 RVTTKHAFAT LSFIAETFLF LYVGMDALDI EKWKFASDSP GKSIGISSIL LGLVLVGRAA 361 FVFPLSFLSN LTKKTELEKI SWRQQIVIWW AGLMRGAVSI ALAYNKFTRS GHTQLHGNAI 421 MITSTITVVL FSTMLFGILT KPLIRFLLPA SSNGAASDPA SPKSLHSPLL TSQLGSDLEA 481 PLPIVRPSSL RMLITKPTHT IHYYWRKFDD ALMRPMFGGR GFVPYSPGSP TDPNVLVE

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27	31580736	AAP55209	Na+/H+ antiporter *Triticum aestivum*	1 MGLDLGALAL KYTGLAVSDH DSIVAINIFI ALLCGCIVFG HLLGGNRWVN ESTAALVLGL 61 ITGGVILICT KGVNSRILIF SEDIFFIYLL PPIIFNAGFQ VKKKQFFRNF ATIILFGAAG 121 TLISFVIITF GAMGLFSKLD VGPLELGDYL AIGAIFSATD SVCTLQVLNQ DEAPLLYSLV 181 FGEGVVNDAT SVVLFNAIQN IDINHFDVFG LLQFIGKFLY LFFTSTVLGV AAGLLSAYII 241 KKLCFARHST DREVAIMILM AYLSCMLSML LDLSGILTVF FCGIVMSHYT WHNVTESSRV 301 TTKHTFATLS FIAEIFLFLY VGMDALDIDK WKLASSSPKK PIALSAVILG LVMVGRAAFV 361 FPLSFLSNLS KKESHPKISF NQQVIIWWAG LMRGAVSIAL AYNKFTTSGH TAVRVNAVMI 421 TSTIIVVLFS TMVFGLLTKP LINLLIPPRP GTAADISSQS FLDPLTASLL GSDFDVGQLT 481 PQTNLQYLLT MPTRSAHRVW RKFDDKFMRP MFGGRGFVPF VPGSPIERSV HGPGLLGTVT 541 EAEDRS
28	30172039	AAP20428	Na+/H+ antiporter NHX1 *Zea mays* subsp. *mays*	1 MGLGVVAELV RLGVLSSTSD HASVVSINLF VALLCACIVL GHLLEENRWV NESTALIVGL 61 GTGTVILMIS RGVVIHVLVF SEDLFFFYLL PPIIFNAGFQ VKKKQFFRNF ITITLFGAVG 121 TLISFTVISL GALGLISRLN IGALELGDYL ALGAIFSATD SVCTLQVLSQ DETPFLYSLV 181 FGEGVVNDAT SVVVFNALQN FDITHIDAEV VFHLLGNFFY LFLLSTVLGV ATGLISALVI 241 KKLYFGRHST DREVALMMLM AYLSYMLAEL FALSGILTVF FGCIVMSHYT WHNVTESSRI 301 TTKHAFATLS FLAETFLFLY VGMDALDIDK WRSVSDTPGK SLAISSILMG LVMVGRAAFV 361 FPLSFLSNLA KKTEHEKISW KQQVVIWWAG LMRGAVSMAL AYKKFTRAGH TQVRGNAIMI 421 TSTIIVVLFS TMVFGLLTKP LINLLIPHRN ATSMLSDDSS PKSLHSPLLT SQLGSDLEEP 481 TNIPRPSSIR GEFLTMTRTV HRYWRKFDDA FMRPMFGGRG FVPFVPGSPT ERNPPDLSKA
29	30172041	AAP20429	Na+/H+ antiporter NHX2 *Zea mays* subsp. *mays*	1 MGLGVDAETV RLGVLSSTSD HASVVSNNFF VALLCACIVL GHLLEENRMV NESITALLVG 61 LGTGTVILMI SRGVSIHVLV FSEDLFFIYL LPPIIFNAGF QVKKKQFFRN FITIILFGAI 121 GTLISFVIIS LGAMGLFKKL DVGPLELGDY LAIGAIFSAT DSVCTLQVLN QDETPLLYSL 181 VFGEGVVNDA TSIVVFNALQ NFDITHINAE VVFHLLGNFL YLFLLSTVLG VATGLISALV 241 IKKIYFGRHS TDREVALMML MAYLSYMLAE LFALSGILTV FFGCIVMSHY TWHNVTESSR 301 ITTKHAFATL SFLAETFIFL YVGMDALDIE KWRSVSDTPG KSIAISSILM GLVMLGRAAF 361 VFPLSFLSNL AKKNEHEKIS WKQQVVIWWS GLMRGAVSMA LAYNKFTRAG HTEVRGNEIM 421 ITSTITVVLF STVVFGLLTK PLIRLLMPHR HLTMLSDDST PKSLHSPLLT SQLGSSIEEP 481 TQIPRPTNIR GEFTTMTRTV HRYWRKFDDK FMRPMFGGRG FVPFVPGSPT ERNPHDLSKP
30	32396168	AAP20430	Na+/H+ antiporter NHX3 *Zea mays* subsp. *mays*	1 MSIGLTAETV TNKLASAEHP QVVPNSVFIA LLCLCLVIGH LLEENRWVNE SITAILVGAA 61 TGTVILLISK GKSSHILVFD EELFFIYLLP PIIFNAGFQV KKKQFFRNFI TIILFGAIGT 121 LISFVIISLG AMGLFKKLDV GPLELGDYLA IGAIFSATDS VCTLQVLNQD ETPLLYSLVF 181 GEGVVNDATS VVLFNAVQKI DFEHLTGEVA LQVFGNFLYL FSTSTVLGIA TGLITAFVLK 241 TLYFGRHSTT RELAIMVLMA YLSFMLAELF SLSGIITVFF CGVLMSHVTW HNVTESSRIT 301 SRHVFAMLSF IAETFLFLYV GTDALDFTKW KTSSLSFGKS LGVSSVLLGL VLVGRAAFVF 361 PLSFLSNLSK KHPGEKITIR QQVVIWWAGL MRGAVSIALA FNKFTRAGHT QVRGNAIMIT 421 STIIVVLFST VVFGLLTKPL INLLIPHRNA TSMLSDDSSP KSLHSPLLTS QLISSIEEPT 481 QIPRPTNIRG EFMTMTRTVH RYWRKFDDKF MRPMFGGRGF VPFVPGSPTE RSSPDLSKA

SEQ ID No	PROTEIN NUMBER (GI)	PROTEIN ACCESSION	PROTEIN DESCRIPTION (SPECIES)	SEQUENCE
31	32396170	AAP20431	Na+/H+ antiporter NHX4 *Zea mays* subsp. *mays*	1 MGYQVVAAQL KLASSADHAS VVIITLFVAL LCACIVLGHL LEENRWLNES ITALIIGLGT 61 GVVILLISRG KNSRLLVFSE DLFFIYLLPP IIFNAGFQVK KKQFFRNFMT ITLFGAVGTM 121 ISFFTISLGA IATFSRMSIG TLDVGDFLAI GAIFSATDSV CTLQVLHQDE TPFLYSLVFG 181 EGVVNDATSV VLFNAVQKIQ FTHINAWTAL QLIGNFLYLF STSTLLGIGT GLITAFVLKK 241 LYFGRHSTTR ELAIMILMAY LSYMLAELFS LSGLLTVFFC GVLMSHVTWH NVTESSRTTS 301 RHVFATLSFI SETFIFLYVG MDALDFEKWK TSSLSFGGTL GVSGVLMGLV MLGRAAFVFP 361 LSFLSNLAKK HQSEKISFRM QVVIWWAGLM RGAVSMALAL NKFTRSGHTQ LHGNAIMITS 421 TITVVLFSTM VFGMITKPLI RLLLPASGHP RELSEPSSPK SFHSPLLTSQ QGSDLESTTN 481 IVRPSSLRGL LTKPTHTVHY YWRKFDDALM RPVFGGRGFV PFVPGSPTER NPPDLSKA
32	32396174	AAP20432	Na+/H+ antiporter NHX5 *Zea mays* subsp. *mays*	1 MSMGYQVVAA QLKVASSADH ASVVIITLFV ALLCACIVLG HLLEENRWLN ESITALIIGL 61 CTGGVILMTT KGKSSHVLVF SEDLFFIYLL PPIIFIAGFQ VKKKQFFRNF MTITLFGAVG 121 TMISFFTISL GAIAIFSRMN IGTLDVGDFL AIGAIFSATD SVCTLQVLHQ DETPFLYSLV 181 FGEGVVNDAT SVVLFNAVQK IQITHINAEV ALQVFGNFLY LFSTSTLLGI ATGLITSFVL 241 KKLYFARHST TRELAIMMLM AYLSYMLAEL FSLSGILTVF FCGVLMSHVT WHNVTESSRI 301 TSRHVFAMLS FIAETFIFLY VGTDALDFDK WKTSSLSFGG TLGVSALIMA LVLLGRAAFV 361 FPLSVLTNFS NKHENESITF KHQVIIWWAG LMRGAVSIAL AFKQFTYSGV TLDPVNAAMV 421 TNTTIVVLFT TLVFGLLTKP LIRLLMPHRH LTMLSDDSTP KSLHSPLLTS QLGSDLEEPT 481 NIPRPSSIRG EFLTMTRTVH RYWRKFDDAF MRPMFGGRGF VPVVPGSPIE RSVPQWSEEA 541 HNKEP
33	32396176	AAP20433	Na+/H+ antiporter NHX6 *Zea mays* subsp. *mays*	1 MGLGVVAELV RLGVLSSTSD HASVVSINLF VALLCACIVL GHLLEENRWV NESITALIIG 61 LCTGVVILLT TKGKSSHILV FSEDLFFIYL LPPIIFNAGF QVKKKQFFRN FMTITLFGAV 121 GTMISFFTIS LGALGLISRL NIGALELGDY LALGAIFSAT DSVCTLQVLS QDETPFLYSL 181 VFGEGVVNDA TSVVVFNALQ NFDITHIDAE VVFHLLGNFF YLFLLSTVLG VATGLISALV 241 IKKLYFGRHS TDREVALMML MAYLSYMLAE LFALSGILTV FFGCIVMSHY TWHNVTESSR 301 ITTKHAFATL SFLAETFLFL YVGMDALDID KWRSVSDTPG KSLAISSILM GLVMVGRAAF 361 VFPLSFLSNL AKKTEHEKIS WKQQVVIWWA GLMRGAVSMA LAYKKFTRAG HTQVRGNAIM 421 ITSTIIVVLF STMVFGLLTK PLINLLIPHR NATSMLSDDS SPKSLHSPLL TSQLGSDLEE 481 PTNIPRPSSI RGEFLTMTRT VHRYWRKFDD AFMRPMFGGR GFVPFVPGSP TERNPPDLSK 541 A

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34	22902099	AAM54141	Na+/H+ antiporter *Gossypium hirsutum*	1 MVAPQLAAVF TKLQTLSTSD HASVVSMNIF VALLCACIVI GHLLEENRWM NESITALIIG 61 VFTGVIILLT SGGKSSHLLV FSEDLFFIYL LPPIIFNAGF QVKKKQFFRN FITIMLFGAV 121 GTLISCTIIS LGVINFFKEM DIGSLDIGDF LAIGAIFAAT DSVCTLQVLN QDETPLLYSL 181 VFGEGVVNDA TSVVLFNAIQ SFDLVNTSPR ILLEFIGSFL YLFLASTMLG VIVGLVSAYI 241 IKKLYFGRHS TDREFALMML MAYLSYIMAE LFYLSGILTV FFCGIVMSHY TWHNVTESSR 301 VTTKHAFATL SFVAETFLFL YVGMDALDME KWRFVSDSPG TSVAVSAVLM GLVMVGRAAF 361 VFPLSFLSNL AKKSTSEKIS FREQIIIWWA GLMRGAVSMA LAYNQFTRGG HTQLRGNAIM 421 ITSTITIVLF STVVFGLMTK PLIRFLLPHP KPTASMLSDQ STPKSMEAPF LGSGQDSFDD 481 SLIGVHRPNS IRALLTTPAH TVHYYWRKFD NAFMRPMFGG RGFVPFVPGS PTERSEPNLP 541 QWQ
35	30144703	AAP15178	Na+/H+ antiporter *Suaeda maritima* subsp. *salsa*	1 MWSQLSSFFA SKMDMVSTSD HASVVSMNLF VALLCGCIVI GHLLEENRWM NESITALLIG 61 LSTGIIILLI SGGKSSHLLV FSEDLFFIYL LPPIIFNAGF QVKKKQFFRN FITIILFGAV 121 GTLVSFIIIS LGSIAIFQKM DIGSLELGDL LAIGAIFAAT DSVCTLQVLN QDETPLLYSL 181 VFGEGVVNDA TSVVLFNAIQ NFDLTHIDHR IAYRIAFQFG GNFLYLFFAS TLLGAVTGLL 241 SAYVIKKLYF GRHSTDREVA LMMLMAYLSY MLAELFYLSG ILTVFFCGIV MSHYTWHNVT 301 ESSRVTTKHA FATLSFVAEI FIFLYVGMDA LDIEKWRFVS DSPGTSVAVS SILLGLLMVG 361 RALLFSLVFL MNLSKKSNSE KVTFNQQIVI WWAGLMRGAV SVALAYNQFS RSGHTQLRGN 421 AIMITSTITV VLFSTMVFGL LTKPLILFML PQPKHFTSAS TVSDLGSPKS FSLPLLEDRQ 481 DSEADLGNDD EEAYPRGTIA RPTSLRMLLN APTHTVHHYW RRFDDYFMRP VFGGRGFVPF 541 VPGSPTEQST TNLSQRT
36	28201131	BAC56698	Na+/H+ antiporter *Hordeum vulgare*	1 MAFEVVAAQL ARLSDALATS DHASVVSINL FVALLCACIV LGHLLEENRW LNESITALII 61 GLCTGVVILM TTKGKSSHVL VFSEDLFFIY LLPPIIFNAG FQVKKKQFFR NFMTITLFGA 121 VGTMISFFTI SLAAIAIFSK MNIGTLDVSD FLAIGAIFSA TDSVCTLQVL NQDETPFLYS 181 LVFGEGVVND ATSVVLFNAL QNFDPNQIDA IVILKFLGNF CYLFVSSTFL GVFSGLLSAY 241 IIKKLYIGRH STDREVALMM LMAYLSYMLA ELLDLSGILT VFFCGIVMSH YTWHNVTESS 301 RVTTKHAFAT LSFIAETFLF LYVGMDALDI EKWKFASDSP GKSIGISSIL LGLVLVGRAA 361 FVFPLSFLSN LTKKTELEKI SWRQQIVIWW AGLMRGAVSI ALAYNKFTRS GHTQLHGNAI 421 MITSTITVVL FSTMLFGILT KPLIRFLLPA SSNGDPSEPS SPKSLHSPLL TSMLGSDMEA 481 PLPIVRPSSL RMLITKPTHT IHYYWRKFDD ALMRPMFGGR GFVPYSPGSP TDPNVIVA

Seq ID No	Protein Number (GI)	Protein Accession	Protein Description (species)	Sequence
37	27948863	AAO25547	Na+/H+ antiporter *Hordeum brevisubulatum*	1 MGWGLGDPPA DYGSIMAVGL FVALMCICII VGHLLEENRW MNESTTALLL GLGAGTVILF 61 ASSGKNSRLM VFSEDLFFIY LLPPIIFNAG FQVKKKQFFR NFMTITLFAV VGTLISFSII 121 SLGAMGLISR LNIGALELGD YLALGAIFSA TDSVCTLQVL SQDETPFLYS LVFGEGVVND 181 ATSVVLFNAI QNFDLGNFSS LKFLQFIGNF LYLFGASTFL GVASGLLSAY VIKKLYFGRH 241 STDREVAIMM LMAYLSYMLA ELLDLSGILT VFFCGIVMSH YTWHNVTESS RVTTKHAFAT 301 LSFISETFLF LYVGMDALDI EKWKIVSETY SPMKSITLSS IILALVLVAR AAFVFPLSYL 361 SNLTKKTAGE KISIRQQVII WWAGLMRGAV SIALAYNKFA KSGHTQLPSN AIMITSTIII 421 VLFSTIVFGL LTKPLIRLLI PARHLTREVS ALSEPSSPKS FLEQLTVNGP ETDVENGVSI 481 RRPTSLRMLL ASPTRSVHHY WRKFDNAFMR PVFGGRGFVP FVPGSPTESS VPLLAHGSEN
38	29825705	AAO91943	Vacuolar Na+/H+ antiporter *Hordeum vulgare*	1 MGPDLGALAL RYTGLAVSDH DSIVAINIFI ALLCGCIVFG HLLEGNRWVN ESTTAIVLGL 61 ITGGVILLCT KGVNSRILIF SEDIFFIYLL PPIIFNAGFQ VKKKQFFRNF ATIILFGAVG 121 TLISFVIITL GAMGLFRKLD VGPLELGDYL AIGAIFSATD SVCTLQVLNQ DQAPLLYSLV 181 FGEGVVNDAT SVVLFNAIQN IDLNHFDVLV LLQLIGKFLY LFLTSTVLGV AAGLLSAYII 241 KKLCFARHST DREVAIMILM AYLSYMLSML LDLSGILTVF FCGIVMSHYT RHNVTESSRV 301 TTKHTFATLS FIAEIFLFLY VGMDALDIDK WKLASSSPKK PIALSAVILG LVMVGRAAFV 361 FPLSYLSNLS KKESHPKISF NQQVIIWWAG LMRGAVSIAL AYNKYTTSGH TAVRVNAVMI 421 TSTIIVVLFS TMVFGLLTKP LINLLVPPRP GTAADISSQS FLDPLTASLL GSDFDVGQLT 481 PQTNLQYLLT MPSRSVHRVW RKFDDKFMRP MFGGRGFVPF VPGSPIERSV HGPGLLGTVT 541 EAENRS